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TERMINAL ADHESIONS OF SALIVARY GLAND CHROMOSOMES IN DROSOPHILA

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Introduction.—In the salivary gland cells of *Drosophila melanogaster*, five long (*X*, 2*L*, 2*R*, 3*L*, 3*R*) and one short (4) chromosome arms issue from a common chromocentre. In smear preparations, the distal ends, or tips, of all these chromosomes may be either free or may be found adhering to each other in any combination. Disregarding the small fourth chromosome, ten two-by-two combinations of the long chromosome arms are possible. This phenomenon of association of chromosome ends in salivary gland cells has previously been reported by Bauer,[1] and Prokofyeva-Belgovskaya.[2] It was reported by Hinton and Sparrow[3] that some of the possible combinations of chromosome arms involved in terminal adhesions occur with a significantly higher frequency than do others. In the strain (Oregon-*R*) studied by Hinton and Sparrow, four combinations significantly exceeded, and five were significantly below the expectation. Thus a definite non-randomness of types of adhesions was established. It was later found by Hinton and Atwood[4] that the relative frequencies of each of the possible combinations are quite different in two wild-type strains (Oregon-*R* and Swedish-*b*).

Bauer[1] attributed the occurrence of terminal adhesions to "a non-specific mutual affinity of the terminal chromomeres." Prokofyeva-Belgovskaya[2] suggested that their occurrence is due to "the mutual attraction of homologous genes in the distal inert regions of these chromosomes." Neither of these hypotheses now appears entirely adequate in view of the more recent data establishing the non-random occurrence of adhesions. The present data are an attempt to test the effects of a number of factors which may be influential in determining the selective nature of terminal adhesions.

Method.—Terminal adhesions were studied in salivary gland preparations made by the aceto-carmine and aceto-orcein methods. Each slide was

searched as thoroughly as possible in order to record all adhesions present. Only cases in which the two tips were in actual contact, or had material stretched between them, were used. The data for each slide were kept separate, and approximately 100 examples of adhesions were recorded for each stock used. A total of 1060 adhesions has been recorded. Unless otherwise noted all data are from females.

Results and Discussion.—The frequencies of the various combinations of chromosome tips involved in terminal adhesions in two wild-type strains is presented in table 1. The data show that the frequencies of the various combinations differ markedly for the two strains. A chi-square test comparing the two strains shows that the difference cannot be due to sampling error.

A comparison of the types of adhesions on two slides from the same strain and a comparison of individual slides with the total data from that strain indicate that the relative frequencies of combinations remain constant within any one strain. Two slides (from a stock of radius-incompletus pink-peach) having sufficient adhesions to make such a test significant are tabulated in columns 6 and 7 of table 1. A chi-square test comparing the two gives a probability of 0.84 that the difference is due to sampling error. A chi-square test comparing the slide in column 7 with the stock (column 8) of which it is a part gives a probability of 0.85.

That the total incidence of terminal adhesions may vary in different strains is shown in a comparison of the percentage of nuclei containing terminal adhesions in each of two stocks. In the *ri* p^{p} stock, 43.7% of the nuclei show adhesions, while in the *ri* p^{p} stock having an interbrachial inversion, the frequency is 17.3%. Great variation in the frequency of terminal adhesions per nucleus was also found to exist between individuals in the same stock.

The possibility was considered that in a normal uninjured nucleus the free ends of all the chromosomes may lie in contact, and that unequal frequencies of the different types of associations observed in smear preparations may be due to some associations breaking more readily than others. In order to test this possibility, 50 nuclei from 10 individuals were studied in total mount preparations of glands. In 42 of these nuclei, from 1 to 5 identifiable free tips were found. The number of times each tip was found free is as follows: *X*—12, 2*L*—15, 2*R*—17, 3*L*—12 and 3*R*—9. From these data it seems unlikely that an effect of differential resistance to smearing occurs.

Another possibility is that certain chromosome arms in the normal nucleus tend to lie side by side, and, consequently, have a greater opportunity to come in contact and to establish an association of their free ends. To test this, 44 well smeared nuclei from four slides were examined and the arrangement of chromosome bases emerging from the "chromocentre"

was recorded. It was found that the X appeared next to $2L$ 22 times, next to $2R$ 27 times, next to $3L$ 23 times and next to $3R$ 18 times. These data indicate that there is no regularity in the arrangement of chromosomes in the nucleus which can be correlated with adhesion frequencies.

Since the terminal adhesion types differ so widely between Oregon-R and Swedish-b it seemed advisable to cross the two strains and examine the F_1. Swedish-b females were crossed to Oregon-R males, thereby placing the chromosomes of the F_1 in Swedish-b cytoplasm. The relative frequencies of types of adhesions in the F_1 were found to be almost identical with those of Oregon-R (column 3, table 1). This would indicate that it is

TABLE 1

	1	2	3	4	5	6	7	8	9	10
CHROMOSOME COMBINATION	ORE.-R ♀ %	Sw-b ♀ %	ORE.-R/Sw-b ♀ %	ORE.-R/Sw-b ♀ % AUTOSOMES	ORE.-R/Sw-b ♂ % AUTOSOMES	SLIDE $ri\ p^p$ ♀ ACTUAL NOS.	SLIDE $ri\ p^p$ ♀ ACTUAL NOS.	$ri\ p^p$ ♀ %	$ri\ p^p$ IN (3LR) ♀ %	Sw-b TIP DEF. ♀ %
X-$2L$	17.3	14.0	16.8			1	0	2.6	15.0	5.6
X-$2R$	3.1	0.9	5.6			3	6	14.2	2.0	27.8
X-$3L$	17.3	16.8	23.6			0	0	1.8	15.0	24.4
X-$3R$	16.3	5.6	17.9			0	1	2.6	22.0	4.4
$2L$-$2R$	3.1	9.3	3.4	9.3	7.3	3	2	8.0	9.0	4.4
$2L$-$3L$	3.1	24.3	2.2	6.2	16.3	4	2	12.5	11.0	11.1
$2L$-$3R$	5.1	10.3	5.6	15.6	11.7	10	9	23.2	5.0	8.9
$2R$-$3L$	6.1	8.4	3.4	9.3	11.7	0	2	3.5	5.0	3.3
$2R$-$3R$	16.3	2.8	13.5	37.5	38.2	2	3	6.2	5.0	2.2
$3L$-$3R$	12.2	7.5	7.9	21.9	14.7	8	5	26.8	11.0	7.8
Total cases	98	107	89	32	68	31	30	112	100	90

NOTE: Columns 1–3 and 8–10 indicate the terminal adhesions for the ten possible combinations in per cents of the total cases observed in the particular stock tabulated; columns 4 and 5 are in per cents of the total cases involving only autosomes; in columns 6 and 7 the actual number of cases is represented for each category. All totals represent actual observed cases.

the chromosomes rather than the cytoplasm which are accountable for the dominance shown.

In the F_1 males of the Swedish-b and Oregon-R cross, it was found that cases involving the X were rare: X-$2L$—1%, X-$2R$—0%, X-$3L$—6% and X-$3R$—5%, of the total cases (78). However, those cases involving only autosomes show similar relative frequencies to the autosomes in the females from the same cross ($P = 0.47$).

Chromosome length as a factor was studied by altering the length of the two arms of the third chromosome by a homozygous interbrachial inversion. The inversion stock was kindly loaned to us by Dr. H. J. Muller. The inversion (Muller[7]) results in a very long arm (the distal part of $3R$ on

the base of 3*L*) and a short arm (the distal part of 3*L* on the 3*R* base). Since the inversion occurred in a *ri* p^p stock, control data were obtained from an unaltered *ri* p^p stock (column 8, table 1). The data for the inversion (column 9, table 1) show a marked change in the relative frequency of types of associations, but one which cannot be explained in terms of change of length of the arms alone. This does not eliminate chromosome length as a possible factor contributing to the selectivity of terminal adhesions, but suggests that in changing the length of chromosome arms some other change must also have occurred.

The question arises concerning the action of a chromosome tip which has become altered or deficient. It was shown by Dobzhansky[6] in *D. pseudoobscura* that a chromosome which has an apparent terminal inversion (Sex-ratio) may establish terminal adhesions with other chromosomes. To test the effect of an altered tip on the relative frequencies of types of adhesions a homozygous terminal deficiency for the *X*-chromosome of *D. melanogaster* (260–10, Sutton[5]) was used. This deficiency occurred in a Swedish-*b* stock and extends to band 1*A*3 (Bridges' map). The data (column 10, table 1) show that such an *X*-chromosome still shows a high frequency of terminal adhesion, even though it lacks its former tip. But it now tends to form different combinations with the other chromosomes. 3*L* in the deficiency stock shows the specificities that *X* showed in the Swedish-*b* control (column 2, table 1), and *X*, in the deficiency stock, shows entirely new specificities. Among other possibilities it may be suggested that the subterminal layers of the chromosomes have characteristic potentialities for selectivity in adhering to other chromosomes, and that in the terminal deficiency a new layer of these potentialities is exposed. These considerations clearly indicate that specificity factors exist as properties of the ends of chromosomes, and may be altered, but not eliminated by a morphological change in the tip of the chromosome itself.

While collecting data certain rare types of adhesions have been observed. One type is the adhesion of three, rather than two, tips. The following have been recorded:

X-2*L*-2*R*—3 cases	*X*-2*R*-3*L*—3 cases	2*L*-2*R*-3*L*—3 cases
X-2*L*-3*L*—3 cases	*X*-2*R*-3*R*—5 cases	2*L*-2*R*-3*R*—3 cases
X-2*L*-3*R*—3 cases	*X*-3*L*-3*R*—10 cases	2*L*-3*L*-3*R*—5 cases
X-2*L*-4 —1 case		2*R*-3*L*-3*R*—1 case

Cases involving four tips in a single adhesion have also been observed: 2*L*-2*R*-3*L*-3*R*—4 cases, *X*-2*L*-2*R*-3*R*—1 case, *X*-2*L*-3*L*-3*R*—1 case, *X*-2*L*-2*R*-3*L*—1 case. Five cases were noted in which the tip of the small fourth chromosome was involved with the tip of one of the long arms. There are regions along the length of the various arms to which tips sometimes ad-

here. Regions to which a tip has been seen to adhere twice or more are: 2, 3*C* and 9 of *X;* 22*A* of 2*L;* 64 and 67 of 3*L;* and 84 and 98 of 3*R* (Bridges' map).

Data were collected from various wild-type strains of *D. pseudoobscura.* We are greatly indebted to Professor Th. Dobzhansky for the bulk of the *D. pseudoobscura* data. Although the strains were not recorded separately, the very low frequency of certain combinations shows that adhesions are definitely non-random. The data are as follows: *XL-XR*—55 cases, *XL*-II—11, *XL*-III—4, *XL*-IV—16, *XR*-II—36, *XR*-III—11, *XR*-IV—27, II-III—25, II-IV—46 and III-IV—20. Cases involving three and four tips and cases involving the small fifth chromosome were also found. Tips were found adhered to a repeat in the 96 region of IV, 8 times, and in 4 cases the repeat was found adhering to the bases of chromosomes.

From the data it seems possible that: terminal adhesions may be due to properties of chromosome ends which are specific for like properties on other chromosome ends, the frequency of the different associations being dependent upon the number of these factors in common; that these specific factors act simultaneously with a general tendency of salivary chromosome tips to adhere; and that this action may be influenced by mechanical agents such as chromosome length.

Summary.—The end-to-end adhesion of salivary gland chromosomes of *D. melanogaster* and *D. pseudoobscura* has been studied by recording the chromosomes involved in adhesions. It is found that the combinations of chromosomes in adhesions are non-random and that the individual chromosomes behave alike within any one strain. The possibility that the types of adhesions are due to a differential resistance to smearing, or to the arrangement of chromosomes in the nucleus, is found to be remote. In the cross between two wild-type strains the F_1 shows the types of adhesions solely of one of the parental strains. Males are similar to females in the same strain except that the frequency of adhesions involving the *X*-chromosome is lower in males. The study of a stock with a tip deficiency and a stock in which the length of two chromosome arms was changed indicated that the relative frequency of types of adhesions can be changed by altering the structure of a chromosome, and that chromosome length may be a contributing factor to the non-randomness. It is concluded that several factors have been eliminated as possible causes of the selectivity of terminal adhesions, and that an hypothesis that the non-randomness is due to a number of specific factors located at the tips, plus a non-specific tendency to adhere, influenced by chromosome length, seems at present most plausible.

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AN EXPERIMENT ON OLFACTORY CONDITIONING IN DROSOPHILA GUTTIFERA

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The recent work of Thorpe[1] upon olfactory conditioning suggests one way in which an isolation may arise within insect populations and therefore it seems desirable to present further data on this subject. Thorpe has shown that two different types of insects (the ichneumonid parasite *Nemeritis canescens* and the vinegar fly *Drosophila melanogaster*) can be conditioned as larvae to give positive responses as adults to the odors of their larval environments. Thus, for instance, adults of both sexes of *Drosophila melanogaster* will be attracted to the odor of essence of peppermint if their larval lives have been spent in a medium where this substance was present. This is true in spite of the fact that the odor is otherwise repellent to the flies.

The fact that such conditioning gradually disappears, in adults of both species studied, upon removal of the conditioning stimulus suggests one means by which ecological changes can be made by adults in populations where conditioning exists. Such a suggestion is supported by observations (Sturtevant unpublished) upon individuals of a variety of species of *Drosophila* that have been taken from the wild for the purpose of establishing laboratory strains. These flies characteristically are slow to lay eggs and much difficulty is experienced in starting them; however, where a strain can be established no further trouble usually occurs. Of course, olfactory conditioning is not the only obstacle to making such stocks—dietary deficiencies, upset mating habits and so on often being involved.

The experiments to be presented here were conducted as follows under the direction of Dr. A. H. Sturtevant. A strain of a fungus-inhabiting species, *Drosophila guttifera*, was selected from the laboratory stocks and used to establish two sub-strains. One was continued upon the food in which it had been developing in the laboratory (a mixture of molasses, corn meal, cotton seed, agar, moldex, yeast and water). The other was started on a new medium of agar, yeast, moldex, water and commercial mushroom extract.

The usual difficulty occurred in starting the new sub-strain so that sufficient flies were not obtained until after more than one generation. (It should be noted that this circumstance raises the possibility that the results given below are due to genetic rather than to conditioned differences between the sub-strains. Such an explanation, however, seems quite improbable because of Thorpe's findings and the fact that the flies from which the sub-strains were established were highly inbred, being all descended from a single female and maintained as a small laboratory population for many generations before the experiment.) When enough flies of compara-

TABLE 1

NUMBER OF EGGS LAID ON FUNGUS (*F*) AND ON LABORATORY (*L*) MEDIA BY FLIES REARED ON THE FUNGUS MEDIUM

	A		*B*		*C*		*D*		*E**		*F**	
TUBES	6 ♀ : 4 ♂		6 ♀ : 4 ♂		6 ♀ : 3 ♂		2 ♀ : 1 ♂		6 ♀ : 4 ♂		6 ♀ : 4 ♂	
DAYS	*F*	*L*	*F*	*L*	*F*	*L*	*F*	*L*	*F*	*L*	*F*	*L*
1	10	3	6	0	1	0	0	0	0	0	0	0
2	13	0	14	0	29	0	0	0	0	0	0	0
3	36	1	0	7	42	0	0	0	6	0	0	0
4	0	0	65	14	34	5	0	0	8	0	4	1
5	17	3	43	28	87	30	0	0	9	0	0	0
6	37	1	82	20	84	8	12	0	13	0	13	0
7	84	0	119	1	99	27	20	0	16	0	2	1
8	78	0	105	19	16	2	6	0	4	0	2	2
9	69	11	129	61	43	0						
10	102	0	66	0	21	0						
11	119	0	175	8	11	13						
12	38	3	88	0	73	0						
13	45	0	47	15	16	0						
14	48	0	158	0	19	20						
15	52	0	71	0								
16	46	0	106	4								
17	30	0										
18	15	0										
Totals	839	22	1269	177	575	105	38	0	56	0	21	4

Eggs laid on laboratory medium = 9.91%.

* See end of table 2 for explanation.

ble age were available, several glass tubes (averaging seven to ten inches long and three-fourths to one inch in diameter) were prepared. Six females and four males of one or the other sub-strain were placed in each tube (unless otherwise indicated in the tables) along with two small metal trays of food, one at each end of the tube. In one tray laboratory medium was placed and in the other the fungus medium. These trays were exchanged every day for fresh ones and the eggs laid counted under a binocular microscope.

As the only purpose of the present experiment was to determine whether

or not egg-laying was influenced by the larval environment (a fact not brought out by Thorpe for this genus) no attempt was made to carefully control and study other environmental factors. One can see from the data below that the early environment does influence significantly the egg-laying responses of the adult flies. The results agree with the data for *Nemeritis* in that they suggest a strong hereditary response to the ancestral environment, which, however, is significantly weakened by rearing on laboratory food.

TABLE 2

NUMBER OF EGGS LAID ON FUNGUS (*F*) AND ON LABORATORY (*L*) MEDIA BY FLIES REARED ON THE LABORATORY MEDIUM

	A		*B*		*C*		*D*		*E**		*F**	
TUBES DAYS	6 ♀ : 3 ♂ *F*	*L*	6 ♀ : 3 ♂ *F*	*L*	6 ♀ : 4 ♂ *F*	*L*	5 ♀ : 4 ♂ *F*	*L*	6 ♀ : 4 ♂ *F*	*L*	6 ♀ : 4 ♂ *F*	*L*
1	0	0	0	0	0	2	6	8	8	18	27	20
2	1	0	4	0	64	0	27	3	23	18	63	0
3	2	4	2	0	23	18	38	0	58	14	87	11
4	34	17	12	10	100	15	18	29	72	0	26	17
5	67	4	34	24	27	1	65	0	41	7	11	42
6	3	89	76	16	58	10	23	0	0	9	70	0
7	70	44	49	26	66	2	95	6	21	1	4	1
8	41	0	94	3	100	0	41	9	0	36	20	3
9	54	21	123	17	28	11	100	0				
10	42	17	18	24	63	0	59	8				
11	59	16	46	0	26	0						
12	24	1	26	0								
13	20	0	1	3								
14	0	22	55	0								
15	17	8										
16	13	0										
Totals	447	243	540	123	555	59	472	63	223	103	308	94

Eggs laid on laboratory medium = 21.20%.

Difference between eggs laid by the two sub-strains on laboratory medium = 11.29%.

S. E.$_{diff.}$ = ±.896%.

* Fungus used in these tubes uniformly older and with less yeast than that of the other tubes.

A similar experiment on the egg-laying preferences of *Drosophila repleta*, a species of catholic tastes, has been conducted by Suster.[2] His work shows that preferences could be established for decomposing boiled carrots and a mixture of sugar and yeast. Suster offers a Lamarckian explanation of his results which, however, cannot be taken seriously in the light of Thorpe's work.

Thorpe has pointed out the rôle of olfactory conditioning as an isolating mechanism and therefore as an agent capable of influencing the migration factor in the forces determining the gene frequencies of wild populations.[3]

In this connection one should note that the factor of selection pressure will probably also vary where such isolation arises, as well as that of population size, for the populations will be held in somewhat different environments.

It may be noted in closing that here is a physiological factor acting on population mechanics that does not depend upon genetic change for changes in its specificity of action; in this respect it is similar to the homing reaction in salmon[4] and birds,[5] to the conditioned mating preferences of birds[6] and to the reactions of ants toward colony mates.[7]

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*GENETIC CONTROL OF BIOCHEMICAL REACTIONS IN NEUROSPORA**

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From the standpoint of physiological genetics the development and functioning of an organism consist essentially of an integrated system of chemical reactions controlled in some manner by genes. It is entirely tenable to suppose that these genes which are themselves a part of the system, control or regulate specific reactions in the system either by acting directly as enzymes or by determining the specificities of enzymes.[1] Since the components of such a system are likely to be interrelated in complex ways, and since the synthesis of the parts of individual genes are presumably dependent on the functioning of other genes, it would appear that there must exist orders of directness of gene control ranging from simple one-to-one relations to relations of great complexity. In investigating the rôles of genes, the physiological geneticist usually attempts to determine the physiological and biochemical bases of already known hereditary traits. This approach, as made in the study of anthocyanin pigments in plants,[2] the fermentation of sugars by yeasts[3] and a number of other instances,[4] has established that many biochemical reactions are in fact controlled in specific ways by specific genes. Furthermore, investigations of this type tend to support the assumption that gene and enzyme

specificities are of the same order.[5] There are, however, a number of limitations inherent in this approach. Perhaps the most serious of these is that the investigator must in general confine himself to a study of non-lethal heritable characters. Such characters are likely to involve more or less non-essential so-called "terminal" reactions.[5] The selection of these for genetic study was perhaps responsible for the now rapidly disappearing belief that genes are concerned only with the control of "superficial" characters. A second difficulty, not unrelated to the first, is that the standard approach to the problem implies the use of characters with visible manifestations. Many such characters involve morphological variations, and these are likely to be based on systems of biochemical reactions so complex as to make analysis exceedingly difficult.

Considerations such as those just outlined have led us to investigate

TABLE 1

GROWTH OF PYRIDOXINLESS STRAIN OF *N. sitophila* ON LIQUID MEDIUM CONTAINING INORGANIC SALTS,[9] 1% SUCROSE, AND 0.004 MICROGRAM BIOTIN PER CC. TEMPERATURE 25°C. GROWTH PERIOD, 6 DAYS FROM INOCULATION WITH CONIDIA

MICROGRAMS B_6 PER 25 CC. MEDIUM	STRAIN	DRY WEIGHT MYCELIA, MG.
0	Normal	76.7
0	Pyridoxinless	1.0
0.01	"	4.2
0.03	"	5.7
0.1	"	13.7
0.3	"	25.5
1.0	"	81.1
3.0	"	81.1
10.0	"	65.4
30.0	"	82.4

the general problem of the genetic control of developmental and metabolic reactions by reversing the ordinary procedure and, instead of attempting to work out the chemical bases of known genetic characters, to set out to determine if and how genes control known biochemical reactions. The ascomycete *Neurospora* offers many advantages for such an approach and is well suited to genetic studies.[6] Accordingly, our program has been built around this organism. The procedure is based on the assumption that x-ray treatment will induce mutations in genes concerned with the control of known specific chemical reactions. If the organism must be able to carry out a certain chemical reaction to survive on a given medium, a mutant unable to do this will obviously be lethal on this medium. Such a mutant can be maintained and studied, however, if it will grow on a medium to which has been added the essential product of the genetically blocked reaction. The experimental procedure based on this reasoning

can best be illustrated by considering a hypothetical example. Normal strains of *Neurospora crassa* are able to use sucrose as a carbon source, and are therefore able to carry out the specific and enzymatically controlled

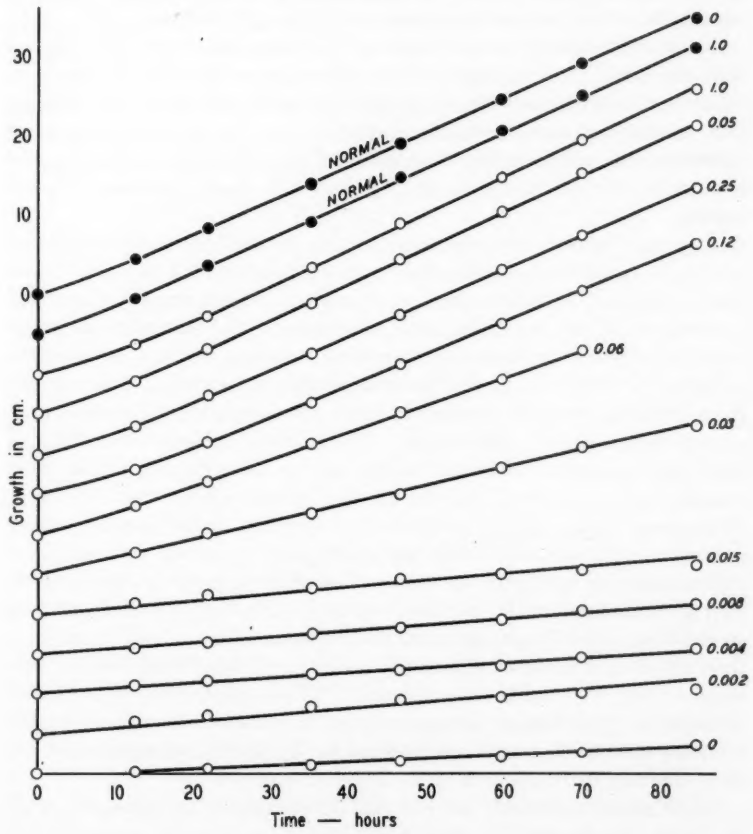

FIGURE 1

Growth of normal (top two curves) and pyridoxinless (remaining curves) strains of *Neurospora sitophila* in horizontal tubes. The scale on the ordinate is shifted a fixed amount for each successive curve in the series. The figures at the right of each curve indicate concentration of pyridoxine (B_6) in micrograms per 25 cc. medium.

reaction involved in the hydrolysis of this sugar. Assuming this reaction to be genetically controlled, it should be possible to induce a gene to mutate to a condition such that the organism could no longer carry out sucrose hydrolysis. A strain carrying this mutant would then be unable to grow

on a medium containing sucrose as a sole carbon source but should be able to grow on a medium containing some other normally utilizable carbon source. In other words, it should be possible to establish and maintain such a mutant strain on a medium containing glucose and detect its inability to utilize sucrose by transferring it to a sucrose medium.

Essentially similar procedures can be developed for a great many metabolic processes. For example, ability to synthesize growth factors (vitamins), amino acids and other essential substances should be lost through gene mutation if our assumptions are correct. Theoretically, any such metabolic deficiency can be "by-passed" if the substance lacking can be supplied in the medium and can pass cell walls and protoplasmic membranes.

In terms of specific experimental practice, we have devised a procedure in which x-rayed single-spore cultures are established on a so-called "complete" medium, i.e., one containing as many of the normally synthesized constituents of the organism as is practicable. Subsequently these are tested by transferring them to a "minimal" medium, i.e., one requiring the organism to carry on all the essential syntheses of which it is capable. In practice the complete medium is made up of agar, inorganic salts, malt extract, yeast extract and glucose. The minimal medium contains agar (optional), inorganic salts and biotin, and a disaccharide, fat or more complex carbon source. Biotin, the one growth factor that wild type *Neurospora* strains cannot synthesize,[7] is supplied in the form of a commercial concentrate containing 100 micrograms of biotin per cc.[8] Any loss of ability to synthesize an essential substance present in the complete medium and absent in the minimal medium is indicated by a strain growing on the first and failing to grow on the second medium. Such strains are then tested in a systematic manner to determine what substance or substances they are unable to synthesize. These subsequent tests include attempts to grow mutant strains on the minimal medium with (1) known vitamins added, (2) amino acids added or (3) glucose substituted for the more complex carbon source of the minimal medium.

Single ascospore strains are individually derived from perithecia of *N. crassa* and *N. sitophila* x-rayed prior to meiosis. Among approximately 2000 such strains, three mutants have been found that grow essentially normally on the complete medium and scarcely at all on the minimal medium with sucrose as the carbon source. One of these strains (*N. sitophila*) proved to be unable to synthesize vitamin B_6 (pyridoxine). A second strain (*N. sitophila*) turned out to be unable to synthesize vitamin B_1 (thiamine). Additional tests show that this strain is able to synthesize the pyrimidine half of the B_1 molecule but not the thiazole half. If thiazole alone is added to the minimal medium, the strain grows essentially normally. A third strain (*N. crassa*) has been found to be unable

to synthesize para-aminobenzoic acid. This mutant strain appears to be entirely normal when grown on the minimal medium to which *p*-aminobenzoic acid has been added. Only in the case of the "pyridoxinless" strain has an analysis of the inheritance of the induced metabolic defect been investigated. For this reason detailed accounts of the thiamine-deficient and *p*-aminobenzoic acid-deficient strains will be deferred.

Qualitative studies indicate clearly that the pyridoxinless mutant, grown on a medium containing one microgram or more of synthetic vitamin B_6 hydrochloride per 25 cc. of medium, closely approaches in rate and characteristics of growth normal strains grown on a similar medium with

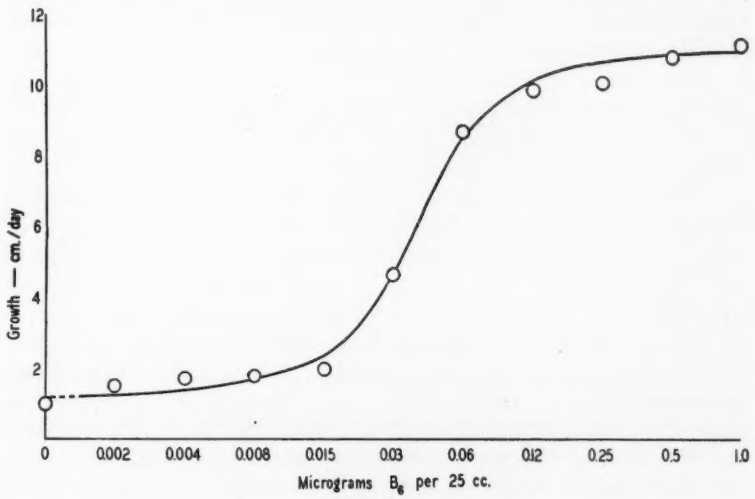

FIGURE 2

The relation between growth rate (cm./day) and vitamin B_6 concentration.

no B_6. Lower concentrations of B_6 give intermediate growth rates. A preliminary investigation of the quantitative dependence of growth of the mutant on vitamin B_6 in the medium gave the results summarized in table 1. Additional experiments have given results essentially similar but in only approximate quantitative agreement with those of table 1. It is clear that additional study of the details of culture conditions is necessary before rate of weight increase of this mutant can be used as an accurate assay for vitamin B_6.

It has been found that the progression of the frontier of mycelia of *Neurospora* along a horizontal glass culture tube half filled with an agar medium provides a convenient method of investigating the quantitative

effects of growth factors. Tubes of about 13 mm. inside diameter and about 40 cm. in length are used. Segments of about 5 cm. at the two ends are turned up at an angle of about 45°. Agar medium is poured in so as to fill the tube about half full and is allowed to set with the main segment of the tube in a horizontal position. The turned up ends of the tube are stoppered with cotton plugs. Inoculations are made at one end of the agar surface and the position of the advancing front recorded at convenient intervals. The frontier formed by the advancing mycelia is remarkably well defined, and there is no difficulty in determining its position to within a millimeter or less. Progression along such tubes is strictly linear with time and the rate is independent of tube length (up to 1.5 meters). The rate is not changed by reducing the inside tube diameter to 9 mm., or by

TABLE 2

RESULTS OF CLASSIFYING SINGLE ASCOSPORE CULTURES FROM THE CROSS OF PYRIDOXINLESS AND NORMAL *N. sitophila*

ASCUS NUMBER	1	2	3	4	5	6	7	8
17	—	*pdx*	*pdx*	*pdx*	*N*	*N*	*N*	—
18	—	—	*N*	*N*	—	—	*pdx*	*pdx*
19	—	*pdx*	—	—	—	—	—	*N*
20	—	—	*N*	—	—	—	—	*pdx*
22	—	—	*N*	—	—	—	—	—
23	—	*	*	*	*N*	*N*	*pdx*	*pdx*
24	*N*	*N*	*N*	*N*	*pdx*	*pdx*	*pdx*	*pdx*

N, normal growth on B_6-free medium. *pdx*, slight growth on B_6-free medium. Failure of ascospore germination indicated by dash.

* Spores 2, 3 and 4 isolated but positions confused. Of these, two germinated and both proved to be mutants.

sealing one or both ends. It therefore appears that gas diffusion is in no way limiting in such tubes.

The results of growing the pyridoxinless strain in horizontal tubes in which the agar medium contained varying amounts of B_6 are shown graphically in figures 1 and 2. Rate of progression is clearly a function of vitamin B_6 concentration in the medium.[10] It is likewise evident that there is no significant difference in rate between the mutant supplied with B_6 and the normal strain growing on a medium without this vitamin. These results are consistent with the assumption that the primary physiological difference between pyridoxinless and normal strains is the inability of the former to carry out the synthesis of vitamin B_6. There is certainly more than one step in this synthesis and accordingly the gene differential involved is presumably concerned with only one specific step in the biosynthesis of vitamin B_6.

In order to ascertain the inheritance of the pyridoxinless character, crosses between normal and mutant strains were made. The techniques for hybridization and ascospore isolation have been worked out and described by Dodge, and by Lindegren.[6] The ascospores from 24 asci of the cross were isolated and their positions in the asci recorded. For some unknown reason, most of these failed to germinate. From seven asci, however, one or more spores germinated. These were grown on a medium containing glucose, malt-extract and yeast extract, and in this they all grew normally. The normal and mutant cultures were differentiated by growing them on a B_6 deficient medium. On this medium the mutant cultures grew very little, while the non-mutant ones grew normally. The results are summarized in table 2. It is clear from these rather limited data that this inability to synthesize vitamins B_6 is transmitted as it should be if it were differentiated from normal by a single gene.

The preliminary results summarized above appear to us to indicate that the approach outlined may offer considerable promise as a method of learning more about how genes regulate development and function. For example, it should be possible, by finding a number of mutants unable to carry out a particular step in a given synthesis, to determine whether only one gene is ordinarily concerned with the immediate regulation of a given specific chemical reaction.

It is evident, from the standpoints of biochemistry and physiology, that the method outlined is of value as a technique for discovering additional substances of physiological significance. Since the complete medium used can be made up with yeast extract or with an extract of normal *Neurospora*, it is evident that if, through mutation, there is lost the ability to synthesize an essential substance, a test strain is thereby made available for use in isolating the substance. It may, of course, be a substance not previously known to be essential for the growth of any organism. Thus we may expect to discover new vitamins, and in the same way, it should be possible to discover additional essential amino acids if such exist. We have, in fact, found a mutant strain that is able to grow on a medium containing Difco yeast extract but unable to grow on any of the synthetic media we have so far tested. Evidently some growth factor present in yeast and as yet unknown to us is essential for *Neurospora*.

Summary.—A procedure is outlined by which, using *Neurospora*, one can discover and maintain x-ray induced mutant strains which are characterized by their inability to carry out specific biochemical processes.

Following this method, three mutant strains have been established. In one of these the ability to synthesize vitamin B_6 has been wholly or largely lost. In a second the ability to synthesize the thiazole half of the vitamin B_1 molecule is absent, and in the third para-aminobenzoic acid is not

synthesized. It is therefore clear that all of these substances are essential growth factors for *Neurospora*.[11]

Growth of the pyridoxinless mutant (a mutant unable to synthesize vitamin B_6) is a function of the B_6 content of the medium on which it is grown. A method is described for measuring the growth by following linear progression of the mycelia along a horizontal tube half filled with an agar medium.

Inability to synthesize vitamin B_6 is apparently differentiated by a single gene from the ability of the organism to elaborate this essential growth substance.

NOTE: Since the manuscript of this paper was sent to press it has been established that inability to synthesize both thiazole and *p*-aminobenzoic acid are also inherited as though differentiated from normal by single genes.

* Work supported in part by a grant from the Rockefeller Foundation. The authors are indebted to Doctors B. O. Dodge, C. C. Lindegren and W. S. Malloch for stocks and for advice on techniques, and to Miss Caryl Parker for technical assistance.

[1] The possibility that genes may act through the mediation of enzymes has been suggested by several authors. See Troland, L. T., *Amer. Nat.*, **51**, 321–350 (1917); Wright, S., *Genetics*, **12**, 530–569 (1927); and Haldane, J. B. S., in *Perspectives in Biochemistry*, Cambridge Univ. Press, pp. 1–10 (1937), for discussions and references.

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[4] See Goldschmidt, R., *Physiological Genetics*, McGraw-Hill, pp. 1–375 (1939), and Beadle, G. W., and Tatum, E. L., *Amer. Nat.*, **75**, 107–116 (1941) for discussion and references.

[5] See Sturtevant, A. H., and Beadle, G. W., *An Introduction to Genetics*, Saunders, pp. 1–391 (1931), and Beadle, G. W., and Tatum, E. L., loc. cit., footnote 4.

[6] Dodge, B. O., *Jour. Agric. Res.*, **35**, 289–305 (1927), and Lindegren, C. C., *Bull. Torrey Bot. Club*, **59**, 85–102 (1932).

[7] In so far as we have carried them, our investigations on the vitamin requirements of *Neurospora* corroborate those of Butler, E. T., Robbins, W. J., and Dodge, B. O., *Science*, **94**, 262–263 (1941).

[8] The biotin concentrate used was obtained from the S. M. A. Corporation, Chagrin Falls, Ohio.

[9] Throughout our work with *Neurospora*, we have used as a salt mixture the one designated number 3 by Fries, N., *Symbolae Bot. Upsalienses*, Vol. 3, No. 2, 1–188 (1938). This has the following composition: NH_4 tartrate, 5 g.; NH_4NO_3, 1 g.; KH_2PO_4, 1 g.; $MgSO_4 \cdot 7H_2O$, 0.5 g.; NaCl, 0.1 g.; $CaCl_2$, 0.1 g.; $FeCl_3$, 10 drops 1% solution; H_2O, 1 l. The tartrate cannot be used as a carbon source by *Neurospora*.

[10] It is planned to investigate further the possibility of using the growth of *Neurospora* strains in the described tubes as a basis of vitamin assay, but it should be emphasized that such additional investigation is essential in order to determine the reproducibility and reliability of the method.

[11] The inference that the three vitamins mentioned are essential for the growth of normal strains is supported by the fact that an extract of the normal strain will serve as a source of vitamin for each of the mutant strains.

THE FREQUENCY OF POLYPLOIDY AND OTHER SPONTANEOUS ABERRATIONS OF CHROMOSOME NUMBER AMONG LARVAE OF THE NEWT, TRITURUS VIRIDESCENS

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Spontaneous deviations from the normal somatic chromosome number have been investigated extensively in populations of plants, partly because of the ease with which the chromosome number of each individual may be determined in root-tip preparations. Comparatively little information is available concerning the range and frequency of such aberrations among animals. Recently, a procedure was described which permits the determination of the chromosome number of living amphibian larvae, in whole mounts of the amputated tip of the tail.[1] In young larvae the fin surrounding the tail is sufficiently transparent to allow the examination of mitotic figures in the large, flattened cells of the epidermis. Furthermore, reliable indirect evidence of polyploidy may be obtained from measurements of the size of the interkinetic nuclei of the epidermis as well as of other tissues of the tailtip which do not contain countable mitotic figures. This secondary evidence is particularly valuable because it reveals the chromosomal constitution of red blood cells and of cells of the lateral line sense organs both of which have moved into the tail from distant regions of the embryo, during earlier stages of development. The tailtip thus offers a truly representative picture of the embryo as a whole.

The tailtip method was originally devised to identify in life the triploid individuals which had been shown by several authors to occur among amphibian embryos in preserved material. The search for triploids among larvae of the newt, *Triturus viridescens*, was almost at once successful and provided material for a study of the effects of triploidy on development.[2,3] During the following three years many more larvae of this species were examined and classified with regard to their chromosome number. In addition to other triploid larvae, a single pentaploid larva was discovered in 1939.[4] The past season, 1940–41, was particularly productive in spontaneous chromosome aberrations. Not only did more triploid and pentaploid individuals appear, but also larvae with other chromosome numbers, both expected and unexpected. These included a haploid,[5] a tetraploid, a hyper-diploid individual with $2n + 2$ chromosomes, a haploid-diploid chimaera[5] and a larva containing both diploid and hyper-triploid cells.

All these individuals with deviating chromosome numbers appeared among larvae which developed from normal untreated eggs; they represent, therefore, natural or spontaneous aberrations. The term "spontaneous"

is preferred for the reason that the larvae were raised in the laboratory from eggs which were laid by pituitary-stimulated females from November to May. Earlier investigations of Kaylor[6] had shown that such "pituitary eggs" behave in all respects like those laid during the breeding season by unstimulated females. This might be expected since the ovaries, following the normal breeding season in April and May, grow rapidly during the summer and, by late fall, contain full-grown oöcytes which are ready for laying and fertilization. However, the possibility still exists that the precocious release of the eggs may favor the production of chromosome abnormalities in some unspecific way. The importance of this factor will be discussed below (see table 2).

The search for aberrant individuals will be continued in the future until a sufficiently large number of larvae will have been investigated to give a reliable index of the range and frequency of spontaneous deviations in this species. However, in view of the considerable number of larvae which have already been examined, a preliminary estimate of the frequencies of various types of numerical aberrations seems to be worth while.

TABLE 1

CHROMOSOME NUMBERS OF LARVAE OF *Triturus viridescens* EXAMINED FROM 1937 TO 1941. DIPLOID NUMBER = 22

Total number of larvae	1074
Diploid	1056
Triploid	10
Tetraploid	1
Pentaploid	3
Haploid	1
Hyper-diploid ($2n + 2$)	1
Haploid-diploid chimaera	1
Diploid-hyper-triploid chimaera	1

A summary of the chromosome numbers of the 1074 larvae which were studied during the last four years is given in table 1. As was to be expected, triploidy is the commonest aberration, with a frequency of about 1%. The triploids must owe their origin to the fairly frequent formation of unreduced gametes. Theoretically, either a diploid egg fertilized by a haploid spermatozoon, or the reciprocal combination, would produce a triploid embryo. In plants, the extra set of chromosomes seems to be of maternal origin in most cases. An instance of paternal origin of two chromosome sets has been described in maize by Rhoades;[7] however, triploidy in this case probably resulted from the union of a haploid egg with two haploid sperms, since crosses between diploid and tetraploid plants have shown that diploid pollen grains, as a rule, do not function normally on a diploid stigma. It has not been possible so far to obtain evidence concerning the origin of the third chromosome set in triploid

Triturus larvae. Earlier observations on frogs by G. and P. Hertwig[8] and by Bataillon and Tchou-Su[9] indicate that, in amphibians also, triploidy arises in the majority of cases from the union of a diploid egg and a haploid sperm.

A low frequency of tetraploidy, less than 1 in 1000, would be expected if it owes its origin to the obviously rare occurrence of a union between two exceptional, unreduced gametes. The astonishingly high frequency of pentaploidy, if it is substantiated by future investigations, would indicate that pentaploidy could hardly be caused by the chance union of a diploid egg, a diploid sperm and a second, haploid sperm, as had been suggested before.[4] While polyspermy is a natural phenomenon in *Triturus viridescens*, all sperm nuclei remain separated from one another because each nucleus is associated with a large aster; and, furthermore, the supernumerary sperm nuclei degenerate early, during the first cleavage mitosis of the diploid fusion nucleus.[10] Another hypothesis seems at present more probable, namely, the occasional suppression of the formation of both polar bodies in an oöcyte; this would leave four sets of chromosomes in the egg and require nothing more to produce pentaploidy than the normal fertilization of such an egg by a haploid sperm.

The presence of one haploid and one partially haploid larva in this population is also of special interest. Haploidy, although easily induced in amphibians by various experimental techniques,[11] had not previously been known to occur spontaneously among untreated larvae. In less than a year, however, the first cases of spontaneous haploidy appeared in three different species of salamanders.[5,12] The hyper-diploid larva also seems to be the first individual of its type to be recorded among normal amphibian larvae. The morphological individuality of the chromosomes has not been studied sufficiently in this species to allow an identification of the two extra chromosomes that are present.

It has been mentioned already that all the larvae used in these investigations developed from eggs laid in the laboratory by pituitary-stimulated females, and that the more or less precocious ovulation of the eggs might conceivably increase their tendency towards chromosomal aberrations. It should be pointed out, however, that triploid amphibian embryos were first discovered in preserved material developed from eggs laid in the spring by unstimulated females, long before the pituitary technique had been introduced. Moreover, a single triploid adult newt of a related species, *Triton taeniatus*, was discovered in nature in Sweden by Böök.[13] Attempts will be made to determine the frequency of triploidy in natural populations of larval and adult salamanders of various species. The chances that the other, less viable types of aberrations could be discovered under natural conditions are very slight.

In view of the possible influence of artificially induced ovulation it is

interesting to compare the frequencies with which polyploidy and other aberrations appeared among our larvae during early winter, late winter and spring. If the artificial release of the eggs is at all an important factor, it would be expected to exert more influence in the early part of the winter than in the spring, just before or during the normal breeding season when the eggs are certainly ready for laying. Table 2 shows, however, that during the season 1940–41 the incidence of chromosome abnormalities was almost as high in March, April and May as it was in November and December. It seems safe, therefore, to assume that the frequencies of different degrees of polyploidy given in table 1 are at least representative of, if not identical with, those present among young larvae in their natural habitat.

With the aid of the tailtip method the occurrence of polyploids has also been studied among larvae of two other species of salamanders, *Triturus*

TABLE 2

FREQUENCY OF NUMERICAL CHROMOSOME ABERRATIONS DURING DIFFERENT PERIODS OF WINTER AND SPRING 1940–41

	NOV.–DEC.	JAN.–FEB.	MARCH–MAY
Total number of larvae examined	162	27	195
Diploid	156	26	190
Total aberrant	6	1	5
Triploid	3	1	1
Tetraploid	1	—	—
Pentaploid	—	—	2
Haploid	1	—	—
$2n + 2$	1	—	—
Haploid-diploid	—	—	1
Diploid-hyper-triploid	—	—	1

pyrrhogaster and *Eurycea bislineata*. In the former species, three triploid individuals and one haploid were found among 273 larvae raised in the laboratory during the past two years.[14,12] In a population of 134 larvae of *Eurycea bislineata* which were investigated in 1938–39, thirteen triploid and two tetraploid individuals were present.[15] The conclusion that this high frequency of polyploidy may be typical for the species has not been substantiated so far by the current investigations of J. V. Michalski which, on the other hand, have demonstrated the occurrence of haploidy and partial haploidy. Apparently, "outbursts" of polyploidy may occur under certain conditions that are not yet understood aside from the generally accepted fact that some matings produce more polyploids than others and that genetic factors may therefore be involved.

In this connection Skalińska's[16] observations on the origin of polyploidy in *Aquilegia* are of interest. She found that repeated selfing of a strain decreased both vigor and fertility of the plants which began to produce

many abortive pollen grains, as well as diploid pollen grains and megaspores in considerable numbers. This increased tendency to polyploid mutations was probably caused by the generally abnormal and retarded development of the plants of this weakened or "senile" strain. It is not improbable that similar physiological factors may also play a rôle in the occasional production of unusually large numbers of polyploid embryos in amphibians.

Little information is available regarding the frequency of spontaneous numerical aberrations in other animals and in plants. In *Drosophila melanogaster*, the frequency of triploidy is probably much less than one per cent. In *Oenothera* cultures, triploidy is not uncommon; the frequency varies between different families and may reach a maximum of 12%.[17] In maize, triploid plants appear with a frequency considerably below one per cent, while not a single spontaneous tetraploid has been recorded so far.[18] On the other hand, haploids appear at a rate of approximately 1 in 2000. The same frequency has been recorded for spontaneous haploidy in *Antirrhinum majus*.[19] As a whole, these very incomplete figures show that numerical chromosome aberrations among salamander larvae are surprisingly abundant.

With the exception of the two chimaeras listed in table 1, the chromosome number seemed to be uniform in each larva studied, as far as could be judged from a comparison of nuclear and cell sizes in sections through different organs and regions of the body. At least, no evidence has been found so far that diploid larvae, for instance, may contain sizable polyploid regions. Partial polyploidy of a very limited extent has been recorded in diploid larvae of *Triturus pyrrhogaster*[14] and *Eurycea bislineata*. In two tailtips of the former species, and in one of the latter, the epidermis of the fin included a small area of tetraploid cells. However, it should be emphasized that the tailfin is a purely larval structure which disappears completely during metamorphosis.

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A WHITE-FLOWERED RACE OF DATURA WHICH IS GENETICALLY DISTINCT FROM SIMILAR WHITE RACES IN NATURE

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Read before the Academy, Monday, October 28, 1940[1]

There are two color forms of *Datura stramonium* found growing in the wild throughout most parts of the temperate regions of the world. One form has purple stems and purple flowers, the other has green stems and white flowers. In all other respects the two forms are identical. In most of the current botanical manuals they are classified as distinct species, the name *D. tatula* being given to the purple-flowered form and *D. stramonium* to the white form. In the herbaceous forms of *Datura* there are only these two flower colors, and in all instances plants with purple stems have purple flowers and those with green stems have white flowers. An exception to this last was observed this summer in an F_2 culture from x-ray treated seed, which segregated for a type with purple stems and white flowers. No breeding work has, as yet, been done on this new type. A white-flowered type has also been extracted from hybrids with *D. ferox*. This type differs from the white forms discussed here, in that the cotyledons and the stem below the cotyledons are purple. This type is therefore indistinguishable in the young seedling stage from the purple-stemmed and purple-flowered forms. The recessive gene responsible for this white-flowered condition has been located in the ·4 half of the 3·4 chromosome.

In 1915 a lot of *Datura* seed was obtained from the Department of Agriculture in Washington. This seed gave both purple-flowered and white-flowered plants. One of the purple-flowered plants became the origin of our standard Line 1, and one of the white-flowered plants was taken as the origin of Line 2. These lines have been continued by selfing for thirty and thirty-one generations, respectively.

Very early in *Datura* investigations it was demonstrated that the two wild forms, purple and white, differ only in a single pair of genes, and that the gene responsible for the purple-flowered (P) form is dominant over that for white flowers (p).[2] We have therefore accepted the two color forms as a

single species and have used the name *stramonium* (Linnaeus, 1753) since it preceded *tatula* (Linnaeus, 1762). It was later shown by the use of $2n + 1$ primaries that the pair of genes responsible for the purple- or white-flowered type is located in the 17·18 (Poinsettia) chromosome.[3] In table 1 are shown the trisomic ratios obtained in male backcrosses of the $2n + 17{\cdot}18$ primary when simplex and when duplex for this gene for white flowers (white-1).

TABLE 1

OFFSPRING OF $2n + 17{\cdot}18$ HETEROZYGOUS FOR WHITE-1

		OFFSPRING PURPLE	OFFSPRING WHITE
white-1 × $2n + 17{\cdot}18$ heterozygous ($PP\dot{p}$)		1958	1040
	Ratio	(2)	(1)
white-1 × $2n + 17{\cdot}18$ heterozygous ($P\dot{p}\dot{p}$)		1166	2392
	Ratio	(1)	(2)

Similarly, female backcrosses of the $2n + 17{\cdot}18$ type heterozygous for purple and white gave the expected trisomic ratios as published earlier. All the other eleven $2n + 1$ primary types gave 1:1, or disomic, ratios in backcrosses. Later records from the $2n + 1{\cdot}18$ (Wiry) type when heterozygous for purple and white, and linkage records with other genes in the 17·18 chromosome show that this gene for white flowers is in the ·18 half of the 17·18 chromosome.

The increase in the rate of mutation, both gene and chromosomal, observed in plants grown from aged seeds has been previously reported.[4, 5, 6] Progenies from plants grown from seed which had been stored under ordinary laboratory conditions for more than four years gave a marked increase in the number of pollen abortion mutations. It was pointed out that these mutations usually occurred as sectors, rarely involving an entire plant, and that one plant might have unmutated sectors as well as one or more different mutated sectors. This is taken to indicate that the mutant sectors originate from initial cells of the embryonic stem which have undergone mutation within the resting (aging) seed.

In the progeny of 315 plants grown from seeds which had been aged from $7\frac{1}{2}$ to $9\frac{1}{2}$ years a total of eleven new morphological types due to mutation have been secured. This is a mutation rate of 3.5 per cent. During the twenty-five years of *Datura* breeding work only nine new morphological or "visible" types have been secured in the progenies of untreated normal parents. It would be very difficult to accurately calculate the rate of mutation in these controls, but it is extremely low. It is also likely that some of these nine mutants may themselves have been caused by the aging of seed. In the same 315 plants from old seed, a total of 30 pollen abortion types (new mutations) had been observed by Dr. Cartledge in earlier tests. This would be a rate of 9.5 per cent. In these tests the mutations which

show as visible morphological effects are thus about one-third as frequent as those which cause pollen abortion.

Among the progenies from plants grown from the seeds which were $7^1/_2$ years old at the time of planting there was one culture which immediately drew our attention. This culture segregated in the seed pan for a green-stemmed type which in all appearances was like Line 2. When these green-stemmed plants matured they bore white flowers and were indistinguishable from Line 2 or from any of the other white-flowered races which had been collected from the wild.

As it seemed likely that these white-flowered plants were the result of some admixture with Line 2 or some other laboratory accident all other selfed capsules which had been harvested from this plant (320546(3)) were immediately planted. Table 2 gives the tabulation of these plantings.

TABLE 2

OFFSPRING OF SELFED CAPSULES OF 320546(3)

CAPSULE	PURPLE	WHITE
A	160	34
*B**	111	25
C	50	..
D	57	..

* Capsule *B* also segregated for "peach" independently of white.

These results indicated that a mutation for white had actually occurred and that it had become established as a sector involving capsules *A* and *B*. This new white-flowered race (white-2) was then crossed with white-1, using Line 2 as the standard white-flowered race. The resulting F_1 plants had purple stems and when mature bore purple flowers. One of these F_1 plants was selfed and among 143 individuals in the resulting F_2 generation there were 84 purple and 59 white seedlings. These figures approximate a 9:7 ratio (80.5:62.4) expected in the F_2 from a cross of two independent factors which produce a similar effect. It was therefore evident that we had obtained, by a mutation presumably caused by some aspect of aging, a new white which was different genetically, but which was not distinguishable in appearance from white-1.

The appropriate steps were taken to locate this new gene for white flowers in a particular chromosome. Table 3 shows the results of male back-crosses of four different $2n + 1$ primaries which were heterozygous for white-2. It will be noted that all but the $2n + 15 \cdot 16$ type give a close approximation to a 1:1 ratio. The $2n + 17 \cdot 18$ type which in table 1 gave 1958*P* to 1040*p* (2:1) when heterozygous for white-1 now gives a perfect 1:1 ratio (129*P* to 129*p*) with white-2. The $2n + 15 \cdot 16$ type is here the primary which gives a 2:1, or trisomic ratio, when simplex for white-2 or a 1:2 ratio when duplex for the gene.

Having located the gene for white-2 in the 15·16 chromosome we undertook to determine which half of the chromosome contained the locus for the gene. As there is only one of the two possible secondaries of the $2n +$ 15·16 type available it was impossible to test for both halves by the use of secondaries. The secondary $2n + 15{\cdot}15$ when heterozygous for white-2 and used as the female in backcrosses gave $92P$ to $71p$ diploid offspring and $29P$ to $20p$ $2n + 15{\cdot}15$ offspring. The nearness of these ratios to a 1:1 ratio, especially in the case of the $2n + 15{\cdot}15$ offspring, is evidence that the locus for the gene for white-2 is not in the ·15 half. It was therefore assumed that the gene must be located in the ·16 half of the 15·16 chromosome. Further breeding tests with tertiaries, or compensating types, which contained the ·16 half as extra material gave positive evidence that the gene is located in the ·16 half. Although the results of tests of linkages with other genes in the 15·16 chromosome are still incomplete the results

TABLE 3

MALE BACKCROSSES OF $2n + 1$ TYPES HETEROZYGOUS FOR WHITE-2

		PURPLE	WHITE
Disomic Ratios			
white-2 × $2n + 5{\cdot}6$ heterozygous	(Pp)	17	16
" × $2n + 17{\cdot}18$ "	(Pp)	129	129
" × $2n + 21{\cdot}22$ "	(Pp)	416	398
	Ratio	(1)	(1)
Trisomic Ratios			
white-2 × $2n + 15{\cdot}16$ heterozygous	(PPp)	829	451
	Ratio	(2)	(1)
" × $2n + 15{\cdot}16$ "	(Ppp)	1022	2050
	Ratio	(1)	(2)

which we have obtained confirm the supposition that the gene for white-2 is in the ·16 half.

Since we now had two genetically different white-flowered types which were indistinguishable from each other in appearance, it seemed desirable to determine whether among the many collections of similar white races from the wild there might not also be more than one gene responsible for the white-flowered condition.

Seed of 1350 races of *Datura* have been assembled since the beginning of our investigations with *Datura*. This assemblage of races has been secured from as widely scattered parts of the world as possible, through our own collections and through the kindness of many correspondents who have sent in seed. The methods used in collecting these samples have been described in an earlier paper.[7] Of these 1350 races, 323 have been classified as belonging to some one of the other nine herbaceous species of *Datura* or to the *Brugmansia* (Tree *Datura*) group. Of the remaining 1027 collections of

D. stramonium 496 have been of the green-stem and white-flowered type; the rest have had purple flowers.

The possibility that there might be more than one gene responsible for white flowers among the many wild races was tested by crossing as many as possible of these white races with standard white testers. If the white flowers of any of these races had been determined by a gene different from that responsible for the white flowers of the standard, the F_1 plants resulting from the cross would have purple flowers. The majority of these crosses were made directly to Line 2 which had been taken as the standard for

TABLE 4

GEOGRAPHICAL DISTRIBUTION OF 409 WHITE RACES OF *D. stramonium* WHICH WERE FOUND TO BE EQUIVALENT TO WHITE-1

ORIGIN	NO. OF RACES TESTED	ORIGIN	NO. OF RACES TESTED
North America		Asia	
United States		China	6
(17 states)	70	Siberia	1
Mexico	4	India	4
West Indies	11	East Indies	8
South America		Iraq	1
Chile	10	Africa	
Brazil	21	Algeria	4
Europe		Belgian Congo	3
England	1	East Africa	2
France	6	West Africa	4
Spain	22	South Africa	6
Switzerland	6	Australia	4
Portugal	7	Botanical Gardens	37
Italy	42	Commercial Sources	5
Hungary	83		
Russia	38		
Germany	3		

white-1. A few of the crosses were made to other white races which in turn had previously been crossed to Line 2 and had been found to be of the white-1 type.

Of the 496 white races available, it was possible to obtain crosses with a standard and the resulting F_1 in 409 cases. All of these F_1 plants had green stems indicating that the white-flowered condition of these races is determined by the same gene as that for white-1. It was not necessary to grow to maturity the F_1 plants of these tests since seedlings of white-1 are entirely green. If any new gene for white had entered into these crosses the resulting seedlings would have been purple-stemmed as were those from the cross of white-1 with white-2.

Table 4 shows from what widely separated parts of the world these 409

white races were secured. In Europe and Asia *D. stramonium* is usually found in the white-flowered form; the reverse of this is true in the United States. This accounts for the larger number of white races tested from Europe. Forty-two races are listed in the table as having been obtained from Botanical Gardens or from commercial sources. The true origin of these from the wild cannot be determined.

In 1937[7] a study was made of the geographical distribution of the chromosomal prime types (*PT*'s) in *D. stramonium*. It was shown that there were only five recurrent prime types among 583 races tested. With the exception of *PT*3 which occurred only in the purple form, all five *PT*'s were found in both purple and white races. Since the summary of 1937 was compiled three white-flowered *PT*3 races from Mexico have been procured. White-flowered races from all five recurrent *PT*'s are included in the present tests with our standard white-1, and in all the gene for white flowers was found to be equivalent to that present in white-1. This is additional evidence that our sample of white races truly represents the wild white-flowered *Daturas*, and is suggestive that the species existed in the white-flowered form before the differentiation into the various *PT*'s occurred.

No other mutation to the green-stemmed or white-flowered condition has ever appeared in all our experiments with *Datura*, which have included x-ray and radium treatments of pollen and seed, heat and cold treatments, injection of chloral hydrate, wounding, and aging of pollen and seed. These treatments have produced many other mutations as evidenced by over 300 new morphological types which have segregated in the F_2 generations. A mutation giving rise to a white type would not have been overlooked as might be possible in the case of a less striking form.

It is not known what the fundamental mechanism may be that brings about genetic changes (mutations) in aged seed. This seed was aged in the laboratory under conditions very different from what would have taken place naturally. It has, indeed, been reported in an earlier paper[8] that *Datura* seed buried in the ground, in a dry condition, for 22 years gave no appreciable increase in the rate of pollen abortion mutations over that found in controls. It may be that the laboratory aging of seed while not directly causing mutation may in some manner make the embryo, or even young seedling, susceptible to some other active mutation-causing agency. That the particular mutation under discussion here must have occurred relatively early is suggested by table 2. Capsules *C* and *D* of the plant within which the mutation to white-1 occurred yielded only purple normal seedlings, indicating that the branches on which they were formed came from initial cells in the seed which had undergone no mutation. Capsule *A* produced purple normal and white normal offspring and so came from an initial cell which had undergone a single mutation. Capsule *B* besides giving purple and white offspring also gave plants of a new type called "peach"

(located in the 13·14 chromosome) on account of the slender and rather pale leaves suggestive of peach leaves. From this capsule there were 88 purple normal, 20 white normal, 23 purple peach and 5 white peach offspring, which is a close approximation of a 9:3:3:1 ratio. It appears in this case that the mutation for white took place fairly early in the embryonic shoot and that somewhere in a succeeding cell carrying the white mutation a second mutation for peach occurred.

Since so many white-flowered races obtained from many widely separate regions of the world, and representing all the recurrent prime types, have been tested by crosses with the standard white-1 and have, in all cases, been determined to be genetically equal to the standard, it is probable that no new gene for the white-flowered condition has become established in the wild. If a mutation to a white-flowered type had occurred in nature there is no evident reason why it should not have survived the processes of natural selection. The white races are in all respects as vigorous and as productive as the purple races. However, we know that natural mutations, or spontaneous mutations from untreated material, are rare in *Datura*. Of the 1350 races of *D. stramonium* which have been assembled eight morphological types differing by a single gene from the standard Line-1 have been recorded. All these have been found to be due to recessive genes, and all have been located in particular chromosomes. As stated above, the rate of mutations from untreated material in our own cultures is also extremely low.

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GENETIC LINKAGE IN THE RABBIT

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The first discovered case of linkage in the rabbit was found by Castle (1924) to exist between albinism and brown coat pigmentation. Later it was shown by Pease (1927) that yellow fat also is linked with albinism. Castle (1933, 1936) then showed that yellow fat is also linked with brown, as was to be expected, the order of the three genes and their map distances apart being *c* 14.4 *y* 28.4 *b*. These are the values given by F_1 females, those found for males being lower as in mice and rats, as follows: *c* 8.2 *y* 26.6 *b*. Interference due to double crossing-over was found to occur in this chromosome, as in *Drosophila* chromosomes, reducing the crossovers between *c* and *b* from a known 42.8 per cent to an apparent 38.3 per cent in females, and from a known 34.8 per cent to an apparent 25.7 per cent in males. The chromosome, in which the three genes, albinism, yellow fat and brown pigmentation lie, may be called Chromosome I of the rabbit.

A second linkage system was shown by Castle (1926) to contain the genes for English and Dutch patterns of white spotting as well as the gene for long (angora) hair. English and Dutch were shown to be very closely linked with each other with less than one per cent of directly observed crossing-over between them, but since Dutch showed a greater frequency of crossing-over with angora than English did, the conclusion was reached that Dutch was more remote from angora, and the order of the genes and their map distances apart were estimated thus:

du 1.19 *En* 13.07 *l*

The chromosome which bears these three genes may be called Chromosome II of the rabbit.

A third linkage system was shown to exist in the rabbit by Castle and Nachtsheim (1933) between two genes for short (rex) hair (r_1 and r_2). The crossover percentage between them was found by Castle (1936) to be 17.2 ± 0.4. They serve as markers of rabbit Chromosome III.

A fourth linkage system was found by Sawin (1934) to involve the gene for agouti hair pattern and a modification of agouti in which the agouti band is unusually wide. The crossover percentage between agouti (*A*) and wide band (*w*) was found to be about 30. To this fourth linkage group may now be added a third mutant gene first described by Greene, Hu and Brown (1934) and designated by them dwarf.* In homozygous dwarf individuals the growth rate is greatly reduced so that the birth weight is less than half that of normal sibs. The dwarfs die of starvation within a few days after

birth because of inability to nurse. Even in heterozygous dwarfs birth weight is reduced by about one third and the ears are shortened, as they are regularly in rabbits of small body size. The gene is thus an incompletely dominant lethal, or semi-lethal. For this reason the usual method of linkage analysis, backcross of the double heterozygote to a double recessive, is impracticable. It is necessary to use, in place of double recessives, animals which are heterozygous for dwarf, though homozygous for the other gene to be tested. Consequently the expected inheritance ratio becomes 3:1:3:1, instead of the usual 1:1:1:1, if no linkage is involved.

Tests of this character have shown no significant departure from expectation in the case of genes *c* (albino) and *En* (English spotting), markers of Chromosomes I and II, respectively. But in the case of the agouti gene (*A*) unmistakable evidence of linkage is obtained. In a backcross population of 304, there were 91 dwarfs, or 29.9 per cent where 25 per cent are expected. There is accordingly no deficiency of dwarfs but rather an excess, which shows that dwarfs are fully viable up to the time of birth.

Since the non-dwarf classes of the backcross population would include both individuals which were heterozygous for dwarf and those which were free from the dwarf gene, but were indistinguishable without a breeding test, it seems best to disregard them and base the calculation of the crossover percentage solely on the dwarf population in which crossover and non-crossover classes are clearly separable. Of the 91 dwarfs, 11 represent recombinations derived from a crossover gamete furnished by the doubly heterozygous parent, whereas the remaining 80 represent non-crossovers. The indicated crossover percentage is thus 12.1 ± 2.3.

The backcross population included two lots, one of which involved an initial coupling cross, the other a repulsion cross. The raw data supplied by these are as follows:

	DwA	*Dw a*	*dw A*	*dw a*	TOTAL
Coupling	84	66	9	53	212
Repulsion	29	34	27	2	92
					304

Dr. E. L. Green has kindly studied these data by the method of maximum likelihood as described by Mather (1938). This method of treatment makes use of the non-dwarf as well as the dwarf classes and leads to the conclusion that the crossover percentage is "most likely" 14.7 ± 2.6, no allowance, however, being made for a possible influence of the sex of the F_1 parent. This figure (14.7) is not very different from that derived from the dwarfs alone (12.1) so we may safely conclude that the crossover percentage is close to 12–15 per cent.

Since, in linkage group IV, genes *A* and *w* are separated by some 30 map

distance units, and *dw* is only 12–15 units from *A*, it follows that *dw* must lie between *A* and *w*, unless it lies on the opposite side of *A* from *w*, in which case the map distance between *dw* and *w* would be about 42–45 units, a not impossible but less probable situation. To decide between these alternatives an investigation of the crossover percentage between *dw* and *w* is necessary and is now in progress.

The existence of linkage in a fifth chromosome (V) between furless and brachydactyl was indicated by studies of J. C. Wightman in Sawin's laboratory. Wightman in an unpublished master's thesis reported negative results from backcross matings involving tests for linkage between brachydactyl and four other mutant genes as follows:

	NON-CROSSOVERS	CROSSOVERS	DEV./P.E.
with English (*En*)	149	154	0.31
with dilution (*d*)	54	40	2.11
with agouti (*A*)	34	35	0.17
with albinism (*c*)	10	11	0.32

In the case of a fifth gene, furless (*f*) he was unable to make backcross matings, but observed in an F_2 of 62 animals the occurrence of only one double recessive combination whereas four would be expected if the two genes lie in different chromosomes. Since at that time facilities were not available for a more extensive test at Brown University, Sawin reported the case to Castle who undertook its investigation by the so-called lethal test method.

Brachydactyl is a character of fair viability, discovered and described by Greene (1935) to whom we are indebted for a foundaton stock of this mutation as well as of dwarf. Its embryology has been studied by Inman (1941). Homozygous furless animals are of low vitality and usually sterile, so it was necessary to employ rabbits heterozygous for furless in the initial cross with brachydactyl. For this cross two F_1 males shown by breeding tests to be carriers of furless (as well as of brachydactyl) were now outcrossed to rabbits carrying neither recessive gene. Four classes of young should result from this cross, all numerically equal, if no linkage exists between furless and brachydactyl, viz., (1) *Ff Br Br*, (2) *FF Br br*, (3) *Ff Br br* and (4) *FF Br Br*. All four classes would be alike (normal) in appearance, differing only in the recessive genes which they carried. It was necessary therefore by a breeding test in the case of each individual to ascertain whether or not it carried either recessive gene. This was accomplished by mating each animal to an F_1 individual known to be a carrier of both genes. A litter of 6 or more young was considered a satisfactory test. Class (1) and class (2) animals would have received a *non-crossover* gamete from the F_1 parent used in the outcross, but class (3) and class (4) animals would have received *crossover* (recombination) gametes. If the

sum of the class (1) and class (2) individuals were significantly greater than the sum of classes (3) and (4), linkage would be indicated. A test has been made of 99 outcross animals distributed in the four classes thus:

(1)	(2)	(3)	(4)
34	37	13	15

The sum of (1) and (2), the non-crossover classes, is 71; the sum of (3) and (4), the crossover classes, is 28, which is 28.3 ± 3.0 per cent of the population, 99. There can accordingly be no doubt that furless and brachydactyl are borne in the same chromosome at an indicated map distance of 28.3 units. They thus constitute linkage group V of the rabbit. It should be noted that this estimate is based solely on the recombination occurring among the gametes produced by F_1 males. In F_1 females the crossover percentage would probably be found to be somewhat greater, possibly 30 or more.

Summary.—Five linkage groups have now been demonstrated in the rabbit. These are genetic markers of 5 of the 22 chromosome pairs. The linkage maps may be diagrammed thus:

I	*c*	*y*	*b*
	0	14.4	42.8
II	*du*	*En*	*l*
	0	1.2	14.3
III	r_1		r_2
	0		17.2
IV	*A*	*dw*	*w*
	0	14.7	30.1
V	*f*		*br*
	0		28.3

* Study of the linkage relations of dwarf, at Brown University, is part of a research program supported in part by a grant from the Rockefeller Foundation.

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FORMATION AND REDUCTION OF INDOPHENOL BLUE IN DEVELOPMENT OF AN ECHINODERM

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The indophenol blue reaction, often called the Nadi reaction, is an oxidation of para-aminodimethylaniline (dimethylparaphenylenediamine) and α-naphthol catalyzed by an intracellular oxidase, "indophenol oxidase," with resulting formation of indophenol blue. Indophenol oxidase is generally believed to play an important rôle in oxidative metabolism and, according to recent work, it is a part of the cytochrome–cytochrome–oxidase system.[1] The reaction has ordinarily been used as an indicator of presence or localization of the oxidase but the concentrations of agents commonly used are highly toxic to living tissues and organisms and kill rapidly. For example, one formula for the Nadi reagent is: α-naphthol 0.144%, para-aminodimethylaniline 0.108%, Na_2CO_3 0.25%. In recent work on echinoderm development Ranzi[2] mixed equal volumes of 1% solutions of the reagents, with KOH added to the α-naphthol. The mixture was added to a drop of sea water containing the echinoderm eggs or embryos in amounts not stated by the author. Concentrations of this order are immediately or very rapidly lethal to developmental stages of echinoderms and, as will appear, show nothing of the conditions in the living animals.

Use of the Method with Living Material.—In experiments with ciliate protozoa, oligochetes and echinoderm and ascidian developmental stages it has been found possible by using the reagents in extremely low concentrations to obtain the reaction in living, normally active organisms. With this procedure intracellular indophenol formation becomes a delicate indicator of certain regional differentials in physiological condition in living embryos and larval stages and their changes in the course of development.

Moreover, with slight decrease of oxygen content of the fluid containing the animals reduction of the intracellular indophenol blue to a colorless form occurs with regional differentials, and color returns with readmission or increase of oxygen. The oxygen decrease is readily brought about by the oxygen uptake of the animals. With non-motile material, e.g., eggs or

early embryos, crowding at the bottom of a layer of fluid a few millimeters deep decreases oxygen content sufficiently in the middle of the group to initiate reduction in a few minutes even when the preparation is open to the air. Motile forms or stages are sealed in small amounts of water containing the reagents, their motor activity serving to keep oxygen content uniform as it decreases. With gradual oxygen decrease in this way regional differences in rate of reduction appear. Reduction and reoxidation can often be repeated several times on the same material but sooner or later the accumulated indophenol in the cells becomes toxic and reduction is retarded or fails to occur, first in the regions which react most intensely. By bringing about reduction at different stages of the reaction it is possible to distinguish the regional differences representing most nearly normal conditions from those resulting from toxic effects. Used in this way with living material the method is doubly valuable as indicating, on the one hand, regional differences and gradients in catalysis of the oxidation resulting in formation of indophenol blue, and, on the other, regional differences and gradients in reduction of the intracellular indophenol blue. In the following account indophenol formation and indophenol reduction are usually referred to as reaction and reduction.

The reaction occurs in living material with extremely minute quantities of the reagents. For example, a concentration of $^1/_{2,560,000}$ of aniline and of $^1/_{3,200,000}$ of α-naphthol in sea water will give the reaction in echinoderm developmental stages, although slowly. With concentrations of this order the color becomes visible in the most rapidly reacting regions in 15 to 30 or 40 minutes, according to temperature and developmental stage. Still lower concentrations will give the reaction provided the volume of solutions is sufficient in relation to volume of material but it has not been regarded as necessary to work with such concentrations. With concentrations forty to fifty times those mentioned above the reaction is much more rapid but the same reaction patterns appear; such concentrations, however, are rapidly toxic or lethal to echinoderm material. Considerable variation in concentration of either reagent does not appreciably affect the resulting pattern unless the concentration becomes high enough to be rapidly toxic. Blastulae, gastrulae and later stages of the sand dollar, *Dendraster excentricus*, the form used in this study, remain motile and normal in appearance in a wide range of the very low concentrations, even after considerable reaction, though there is sooner or later progressive toxic effect, retardation and cessation of movement and death in all cases observed.

Patterns of Reaction and Reduction in Dendraster Development.—The reaction appears as a gradient or gradients in definite relation to developmental pattern and undergoing definite changes in the course of development. From late cleavage and blastula stages to the gastrula the reaction progresses from the apical ("animal") region basipetally, constituting a

basipetal gradient both in time and in depth of color. In the gastrula and probably earlier a ventrodorsal gradation in reaction also becomes evident with ventral region in advance. Within the blastocoel mesenchyme reacts more rapidly than entoderm, but with progress of entodermal invagination an archenteric gradient appears with "high" end at the tip of the entoderm and reactivity apparently increases there as coelom formation begins. In the prepluteus or "prism" stage the apicobasal-ventrodorsal gradient is still visible in the ectoderm. With beginning of growth of the oral lobe from the apical region reactivity of this region increases and as the anal arms begin development the arm regions show increased reactivity; in later stages of arm outgrowth each arm becomes a reaction gradient with high end at the tip. As the ciliated band of the pluteus develops it becomes a region of higher reactivity than the general ectoderm. With the progress of reaction all these gradients become less distinct or disappear completely in more or less uniform coloration if cytolysis with more or less decoloration does not occur. With completion of pluteus development gradient pattern becomes less evident until little of the earlier pattern remains. That the reaction gradients are not simply penetration gradients is readily shown by placing the material in a low concentration of one of the reagents for a short time and then adding the other in sufficient concentration to give the reaction rapidly. Penetration of both agents, even in the low concentrations, is evidently rapid but the reaction gradients appear with wide ranges of concentration. After immigration of primary mesenchyme into the blastocoel it reacts almost or quite as soon as ectoderm although the reagents must pass through the cell wall of the blastula to reach it.

In late cleavage and early blastula, reduction in slightly decreased oxygen progresses basipetally but the gradient is slight. In the later blastula stages there is apparently a rather rapid and considerable change in condition in the basal region. Reduction becomes much more rapid in the prospective entoderm and mesenchyme than in ectoderm and progresses acropetally from the basal region, obliterating the primary basipetal gradient except in the apical half or less where it persists with a slight differential. However, if the reaction is allowed to progress too far this basipetal gradient is completely obliterated and reduction progresses acropetally throughout with more or less retardation in the apical region.

The change in the reduction pattern during the blastula stage coincides with that indicated by reduction of methylene blue and Janus green[3] and suggests alteration of physiological pattern but the oxidative reaction gives no evidence of change. This point is of particular interest since it seems to indicate an alteration in certain aspects of the oxidation-reduction mechanism in the entodermal-mesenchymal region preceding gastrulation. That this change in reduction pattern is not merely the consequence of the

relative increase in thickness of the blastula wall and the greater cell mass in the basal region seems evident from the fact that basal reduction is first evident, not in the interior of the cell mass, but at its inner surface facing the blastocoel. Apparently oxygen content is lower in the blastocoel than outside and with external oxygen decrease the critical oxygen level is reached first in the blastocoel. Moreover, as will appear below, this acropetal reduction pattern may appear in early stages of exogastrulae in which there is little or no difference in thickness of the cell layer.

Patterns of reaction and reduction in later developmental stages are apparently essentially similar though it is perhaps uncertain whether, or to what extent, the observed patterns represent the real differences between ectoderm directly exposed to external environment and the internal entoderm and mesenchyme.

Reaction and Reduction Patterns in Exogastrulation.—Under this head a few points deserve mention as showing how these patterns may be experimentally altered. Exogastrulation results from the action of LiCl and various other differentially toxic agents, all of which inhibit development. The resulting forms constitute continuous series of differential modifications of development, the degree and morphological character of the modification depending on concentration or intensity of action of agent used, length of exposure period, stage of development at which exposure is begun and on secondary modifications resulting from differential tolerance or conditioning to low concentrations or intensities of agent or differential recovery after return to water. Indophenol reaction and reduction patterns differ to some extent according to the part played by these various factors and possible effects on patterns of the indophenol reagents and of the intracellular indophenol must also be kept in mind.

With exposure beginning soon after fertilization to LiCl or some other agent in the range of concentrations or intensities known to be effective in producing exogastrulation the indophenol reaction in blastulae and early exogastrulae is usually basipetal as in early normal blastulae, but much slower. With increasing toxic effect of agent gradient pattern may be entirely obliterated but such forms do not develop further. The reaction progresses from the blastocoel outward, this course being particularly conspicuous in the thick-walled spherical blastulae resulting from relatively high degrees of inhibition. The reduction pattern in the less inhibited later blastulae is much like the normal, being chiefly acropetal from the basal region with a slight basipetal gradient in the apical region. With increasing inhibition the basipetal gradient does not appear and reduction becomes wholly acropetal, or the gradient is obliterated and development stops.

In the less modified exogastrulae in which the ectoderm attains or approaches pluteus form after return to water ectodermal reaction and re-

duction patterns are essentially like those of normal larvae. Entodermal gradient pattern varies in these forms. Large, elongated external entoderms with more or less differentiation of gut regions and representing considerable recovery after return to water usually show reaction progressing from the tip but reduction may progress from the ecto-entodermal junction toward the tip or in some cases from the tip.

In later stages of the more extreme exogastrular modifications in which the original prospective entoderm has undergone dissociation and has become mesenchymal in function, the external entoderm is derived from entodermized ectoderm and the remaining ectoderm is small and radial in form (pseudoexogastrulae[4]) gradient patterns vary according to degrees of primary effect of the inhibiting agent and of secondary modification after return to water. In the radial ectoderms ventrodorsal reaction and reduction gradients are completely absent but a very slight basipetal reaction gradient may be present and recovery in water may result in apical thickening or slight outgrowth representing a rudimentary oral lobe and characterized by increased reaction, that is, traces of the original basipetal reaction pattern remain. When all but the extreme apical ectoderm has been entodermized the small rounded ectodermal sac or vesicle remaining is usually entirely without reaction gradient. Reaction is very slow, slight and apparently uniform. In the large, more or less elongated external entoderm of these forms the entodermal tip is the high end of the reaction gradient. This gradient is the reverse in direction of the gradient in the ectoderm from which this entoderm is derived and apparently represents secondary modification associated with differential recovery following return to water.

Reduction in these forms, like reduction of methylene blue and Janus green,[5] usually progresses in both directions from the ecto-entodermal junction but very small ectoderms are without appreciable reduction gradient and in some individuals with long entoderms showing considerable regional differentiation reduction progresses from the tip. Forms in which the whole ectoderm is entodermized usually remain spherical and apolar and show no gradient patterns. These few data are sufficient to indicate that there is a correlation between the physiological patterns of indophenol reaction and reduction and the degree and character of exogastrular modifications of development.

The Effect of Killing.—When the embryos or larvae are killed by boiling water, alcohol, $HgCl_2$ or by either, or a mixture, of the indophenol reagents the reaction is much less rapid than in living animals and only the merest trace or no evidence at all of the gradient patterns remains. Reduction does not occur in the dead material and when living material approaches death reduction is retarded or fails to occur or animals which have reduced show reoxidation. In this connection it may be noted that Ranzi and

Falkenheim[2] found no evidence of indophenol gradient pattern in echinoderm material and deny its existence. From their account of concentrations used the material must have been killed at once and the gradient pattern destroyed.

Conclusion.—From the data briefly presented it seems evident that the physiological patterns indicated by indophenol reaction and reduction gradients are characteristic features of echinoderm development, that they can be experimentally altered in steepness and length, reversed in direction or completely obliterated in living animals and that such alterations are correlated with modifications in the course of development. That the morphological modifications are expressions of alterations in these and probably other features of physiological pattern not shown by this method seems probable.

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THE PROVITAMIN A CONTENT OF AMERICAN WHOLE WHEAT FLOUR AND WHOLE WHEAT BREAD

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It has been shown on the basis of chromatographic experiments[1] carried out in collaboration with Dr. Cholnoky[2] that unbleached fine-grade wheat flour originating in Hungary contains only traces of carotene. Therefore it is valueless as a provitamin A source, contrary to numerous older and some newer claims. In spite of the fact that Scheunert and Schieblich[3] reported four years ago that flour is unable to cure vitamin A deficiency in rats, a possible rôle of the flour carotene in human nutrition is still not generally denied. As late as 1939 the Council on Foods of the American Medical Association[4] discussed some data of Copping,[5] on the basis of which a one pound loaf made of unbleached flour would furnish as much as 0.6 to 1.2 mg. of carotene. Binnington, Hutchinson and Ferrari[6] confirm, however, our viewpoint by the statement that "recent unpublished studies

. . . indicate that the major carotenoid pigment is xanthophyll or its esters, rather than carotene itself." Finally an Editorial of the *Journal of the American Medical Association*[7] concludes that the repetition of our experiments with various milling products is desirable.

Such analyses have now been carried out with freshly milled, unbleached American whole wheat flours. The spectra of even crude total extracts indicate that the main constituent of the lipoid soluble pigment is xanthophyll (lutein), the maxima being, e.g., at 477.5 and 448.5 mμ in petroleum ether or at 506 and 473 mμ in carbon disulphide. The pigment content of the saponified extracts varied between 1.5 and 2.0 mg. per kilo flour. It can be roughly divided in the Tswett column into a main xanthophyll fraction and a more weakly adsorbed smaller portion. The latter does not exceed 1–2% of the total pigment and shows the spectrum of β-carotene, of which it mainly consists. In exceptional cases the chromatographic filtrate was also pale yellowish but only because of unsatisfactory saponification of some xanthophyll esters; it was free of carotene.

On the basis of the present estimations the total amount of provitamin A in the flour is 0.02–0.04 mg. per kilo table 1). Cryptoxanthin is not present.

Considering the length of the procedure, the use of strong alkali, the sensitivity of polyenes and the presence of comparatively huge amounts of colorless accompanying material, it was necessary to ascertain that no substantial losses had occurred during the analysis. Therefore 0.1 mg. of crystalline β-carotene was dissolved in the total extract freshly obtained from 1 kilo of flour and all usual procedures (including saponification) were carried out. Simultaneously a blank experiment was run with another kilo of the same flour. The subsidiary layers of the two columns differed visibly; half of the added carotene was found, recovered and estimated. When, however, in two analogous experiments only 0.01 mg. of carotene was added, 0 and 25% of the latter was recovered. Obviously the limits of reliability of the method have been reached here.

We believe that the true carotene content of whole wheat flour is considerably less than 0.1 mg. per kilo, but it cannot be decided if it is nearer 0.02 or 0.06 mg. The A-requirements of an adult person would be covered by 50–100 kilos of flour daily.

Since in some older papers the point seems to have been missed that not flour- but bread-carotene should be considered from the nutritional viewpoint, 100% whole wheat bread was also investigated. If no pigment losses occurred during the baking process, the carotenoid content of the loaf would be near 60% that of the flour, i.e., 0.9–1.2 mg. total pigment and 0.01–0.04 mg. carotene per kilo. On the basis of table 2, however, which presents some data for dried crust and crumb separately, the corresponding true figures appear as follows: 0.26 mg. total pigment and 0.005 mg. of β-

TABLE 1

WHOLE WHEAT FLOUR CAROTENOIDS

(The spectra refer to petroleum ether solutions in which pure lutein shows maxima at 477.5, 448 mμ, pure β-carotene at 484.5, 452.5 mμ.)

NO.	TYPE OF WHEAT	COMMERCIAL DESIGNATION OF THE FLOUR	PIGMENT IN THE TOTAL EXTRACT		PIGMENT IN THE XANTHOPHYLL FRACTION		PIGMENT IN THE CAROTENE-CONTAINING FRACTION	
			MG./KG.	SPECTRUM (mμ)	MG./KG. (MINIMUM FIGURES)	SPECTRUM (mμ)	MG./KG.	SPECTRUM (mμ)
1	California White Wheat	Local Entire	2.0	477.5 447.5	1.4	478 448	0.04	484 452.5
2	California White Wheat	Local Entire	1.9	477 447	1.3	477 448	0.04	484 452.5
3	Dark Northern Spring Wheat	Montana Entire	1.6	478.5 448.5	1.0	478 448	0.02	485 454.5
4	Dark Northern Spring Wheat	Montana Entire	1.5	478 448.5	1.1	478.5 449	0.01	485 453.5
5	Dark Hard Winter Wheat	Eastern Entire	1.9	477 447	1.4	477.5 447	0.01	485 452.5

carotene per kilo of fresh bread or roughly the half of this amount in a commercial pound loaf.

Experiments with *rye* flour revealed the presence of 2–3 mg. of total carotenoids and of 0.03–0.06 mg. of β-carotene per kilo; a sample of pumpernickel bread contained $^1/_{15}$ of these amounts.

EXPERIMENTAL

Small Scale Experiments with Flour.—One kilo of flour was percolated with peroxide-free ether for about three hours. The extracts were bright yellow and showed a grayish blue fluorescence in ultra-violet light. The crude extract (1 liter) was saponified over night with methanolic KOH. Half a liter of water was cautiously added, the alkaline solution reëxtracted with ether and the combined ether solution washed alkali free, dried with sodium sulfate and evaporated *in vacuo*, under CO_2. This evaporation was repeated twice after the addition of small amounts of petroleum ether (b. p. 60–70°). The oily, darkish residue was dissolved in 25 cc. of the solvent mentioned, and chromatographed on a calcium hydroxide column (Shell) (18 × 3.5 cm.). After the column was developed, a 30–40 mm. broad yellow zone (xanthophylls) was visible near the top and about 70 mm. deeper a carotene-containing pale double line was noticed (0.5 and 2 mm. thick).

The xanthophylls were eluted with ether which was then evapo-

rated. It is advisable to eliminate the last traces of this solvent by adding some petroleum ether and distilling it off *in vacuo*. The residue is dissolved in petroleum ether. The carotene layers were eluted with a 1:2 alcohol-petroleum ether mixture, washed free of alcohol and dried. All photometric values were taken in the Pulfrich gradation photometer (Zeiss, light filter S-47, photometric values as found by Cholnoky (in press)). The spectra were determined in an Evaluating Grating Spectroscope as devised by Loewe and Schumm (Zeiss, Jena light filters BG-7) (table 1). The identity of carotene was repeatedly established by the fact that the main portion of the subsidiary layers did not separate from added β-carotene in the mixed chromatogram.

Large Scale Experiments with Flour.—A series of large scale experiments carried out with 25–40 kilos followed in principle the method described above. Five-kilo portions of the flour were extracted in percolators, the

TABLE 2

CAROTENOIDS IN 100% WHOLE WHEAT BREAD

(The weight ratio crust: crumb was 1:3 in the fresh bread. Dry weights: crust 75%, crumb 65%.)

No.	MATERIAL (DRIED)	PIGMENT IN THE TOTAL EXTRACT			PIGMENT IN THE XANTHOPHYLL FRACTION			PIGMENT IN THE CAROTENE-CONTAINING FRACTION		
		MG./KG.	SPECTRUM (Mμ)		MG./KG. (MINIMUM FIGURES)	SPECTRUM (Mμ)		MG./KG.	SPECTRUM (Mμ)	
1*a*	Crust	0.28	478	447.5	0.18	477	446.5	0.005	485	453
2*a*	Crust	0.32	477.5	446.5	0.20	476	446.5	0.005	484.5	453.5
1*b*	Crumb	0.39	476.5	447	0.29	477	447	0.005	484	451.5
2*b*	Crumb	0.38	477.5	447.5	0.32	477	446.5	0.01	483	451.5

weaker solutions being used for the next portion. About $^1/_2$ liter of ether per kilo material was sufficient. Since the saponified solutions as used in this work were generally much more concentrated than those in the analytical experiments, large amounts of sterols and other colorless contaminants had to be eliminated. The latter were partially fluorescent and hence observable on the column in ultra-violet light. Near 0° the most highly concentrated pigment solutions yielded abundant quantities of white crystals which were repeatedly centrifuged off in the cold room and washed with ice cold petroleum ether. The crystals can also be precipitated from light petroleum by alcohol at 0°. In this way it is easy to isolate $^1/_2$ gram or more of long colorless crystals per kilo flour. Without such purifications the high viscosity of some xanthophyll-containing solutions may delay the passing through the column and even obstruct it.

All layers were re-chromatographed several times on lime, the xanthophylls finally on calcium carbonate. In the course of these operations

spontaneous pigment crystallization from petroleum ether repeatedly occurred and the crystals could in each case be identified as lutein.[2] The carotene-containing layers were submitted to mixed chromatography with β-carotene originating from another source; the larger subsidiary layer showed identity.

The details of the lengthy chromatographic fractionations of the xanthophylls can be omitted here. All total xanthophyll layers were heterogeneous but it was impossible to separate any well-defined carotenoid reliably different from lutein. Some layers having lower spectra than lutein (e.g., 472.5, 442.5 mμ in petroleum ether) were partially reconverted into the latter by heating and must thus be qualified as neo-luteins formed during the operations.[8] Other fractions showed approximately the maxima of lutein (e.g., 478.5, 450 mμ or 476.5, 446 mμ). Only in few cases was a higher spectrum found (481, 449 mμ) but the respective zones were very poor in dye.

Bread.—The crust and crumb samples were dried over night at 45°, milled, extracted in 1–2.5 kilo portions with ether and worked up as described above for small scale experiments (see table 2).

Summary.—The carotene content of some freshly milled, unbleached American whole wheat flours is, as estimated by means of the Tswett chromatographic adsorption method, of the order of magnitude of 0.02 to 0.06 mg. β-carotene per kilo. Whole wheat bread is even poorer in carotene. Thus wheat flour and bread are of no importance as provitamin A sources in human nutrition.

The above results seem to be in accordance with a paper of Pulkki and Puutula[9] of which only an abstract was available to the authors.

* Contribution No. 846.

[1] Cf. Zechmeister, L., and Cholnoky, L., *Principles and Practice of Chromatography*, Chapman and Hall, London; John Wiley and Sons, Inc., 440 Fourth Ave., New York, 1941.

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METRIC CONDITIONS FOR SYMMETRIC FINSLER SPACES

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A Finsler Space is a coördinate manifold $\mathfrak{M}$ which carries a locally Minkowskian Metric. This metric determines (or is determined by) a function $F(x,\lambda)$ defined on all contravariant vectors (x, λ) on $\mathfrak{M}$. If $\mathfrak{M}$ and F satisfy the usual conditions, any two points A,B of $\mathfrak{M}$ can be connected by an arc which minimizes $\int F(x, \dot{x})\, dt$ among all curves of class D' from A to B.

Let $F(x, \lambda) = F(x, -\lambda)$. With

$$AB = \int_c F(x, \dot{x})\, dt, \tag{1}$$

where c is a minimizing arc from A to B, the manifold $\mathfrak{M}$ becomes a finitely compact, convex, metric space. Sufficiently short minimizing arcs can be prolonged. In terms of the metric AB this can be formulated as

D: *Every point P of $\mathfrak{M}$ has a neighborhood ν with this property: For any two points $A \neq B$ in ν and a given $\epsilon > 0$ there is a positive $\delta(A, B, \epsilon) \leq \epsilon$ to which there corresponds a unique point B_δ with*

$$AB + BB_\delta = AB_\delta \text{ and } BB_\delta = \delta.$$

If one requires a set of axioms for a metric space, whose geodesics have the "usual" properties, D seems to be just the right condition. Namely, it is not hard to prove:

I. *For every point P of a finitely compact, convex, metric space in which D holds there exists a number $\rho(P) > 0$ such that any two points A, B of $XP < \rho$ may be connected by exactly one segment $\overline{AB}$. $\overline{AB}$ can be extended beyond B (or A) to a segment of length 2ρ, and this extension is unique.*

The extended segment will be denoted by $\overrightarrow{AB}$ ($\overleftarrow{AB}$). Naturally we define a *geodesic* in a metric space as a locally isometric image $t \to P(t)$ of the real axis $-\infty < t < \infty$, more explicitly: for every t there must exist an $\epsilon(t) > 0$ so that

$$P(t_1)P(t_2) = |t_2 - t_1| \text{ for } |t_i - t| < \epsilon(t), \quad i = 1,2.$$

We now have the theorems

II. *Every segment α in a finitely compact, convex, metric space which satisfies D is contained in one and only one geodesic g.*

(This is to be understood in the wider sense, that g is the only geodesic which contains all points of α, even if we disregard the order in which the points of α occur on g.)

III. *If under the assumptions of II the space is one- or two-dimensional (in the pointset theoretical sense) it is a manifold.*

The one-dimensional case is trivial. In the two-dimensional case one may show that the axiom of Pasch holds locally.[1] The corresponding question for more dimensions is open and probably inaccessible.

Consider again the case of a manifold $\mathfrak{M}$ with a metric AB defined by $F(x, \lambda)$. Take a definite coördinate system (x), and let x_0 be a fixed point in the neighborhood covered by these coördinates. Then $F(x_0, x - y) = m(x, y)$ is a Minkowski metric, which is topologically equivalent to the metric (1) and which approximates (1) at x_0 in this sense:

$$\frac{m(x_\nu, y_\nu)}{x_\nu y_\nu} \to 1, \text{ if } x_\nu \to x_0,\ y_\nu \to x_0 \text{ and } x_\nu \neq y_\nu. \tag{2}$$

Furthermore the geodesics have the following property:

Δ: *If the points A_ν, B_ν, C_ν tend to P in such a way that the segments $\overrightarrow{A_\nu B_\nu}$ and $\overrightarrow{A_\nu C_\nu}$ converge, but to different limits, and if $\lim \frac{A_\nu B_\nu}{A_\nu C_\nu}$ exists, then the segments $\overrightarrow{B_\nu C_\nu}$ converge and the equation*

$$\frac{B_\nu A_\nu + A_\nu C_\nu}{B_\nu C_\nu} \to 1$$

holds if, and only if, $\lim \overrightarrow{B_\nu A_\nu} = \lim \overrightarrow{B_\nu C_\nu}$ *or* $\lim \overrightarrow{A_\nu C_\nu} = \lim B_\nu C_\nu$. (The last part of Δ is another formulation of the regularity of $F(x, \lambda)$ at P.)

A priori Δ and the existence of a Minkowski Metric $m(x, y)$, which approximates xy at x_0 do not seem closely related. However, we have:

IV. *A finitely compact, convex, metric space which satisfies conditions D and Δ, is a coördinate manifold of some finite dimension. The topological mapping $x \to P(x)$ of a neighborhood ν of P onto $|x| = \left[\sum_{i=1}^{n} x_i^2\right]^{1/2} < \delta$ can be chosen such that*

1. The geodesic arcs through P in ν go into the open Euclidean segments in $|x| < \delta$ through $(0, 0 \ldots, 0)$ (i.e., the x_i are *normal coördinates*).
2. If $|\lambda| = 1$ then

$$F_P(\lambda) = \lim_{t \to 0} \frac{P(t\lambda)}{|t|}$$

exists.

3. Setting $F_P(0) = 0$ and $F_P(x) = |x| F\left(\frac{x}{|x|}\right)$ for $x \neq 0$ the number $F_P(x - y)$ defines a *Minkowski Metric* with a strictly convex unit sphere which has 0 as center (in the Euclidean sense, i.e., $F_P(x) = F_P(-x)$).
4. $F_P(x - y)$ is topologically equivalent to xy in ν and is an *approximation at P of the original metric:*

$$\frac{F_P(x^\nu - y^\nu)}{P(x^\nu)P(y^\nu)} \to 1, \text{ when } x^\nu \to 0,\ y^\nu \to 0 \text{ and } \frac{|x^\nu| + |y^\nu|}{|x^\nu - y^\nu|} < M < \infty.^2$$

The main point in IV is, of course, the existence of coördinate neighborhoods. Since almost nothing is known concerning topological characterizations of the E^n, the proof is anything but straightforward. If A is different from, but sufficiently close to P, there is exactly one point A_t, $0 \leq t \leq 1$, with $PA_t + A_tA = PA$; put $P_t = P$. It follows from Δ that

$$m(A, B) = \lim_{t \to 0_t} \frac{A_tB_t}{t}$$

exists. One may show with the help of Δ that in a certain neighborhood $XP < \rho$ the function $m(A,B)$ is a metric equivalent to AB, and that

$$\frac{m(A_\nu, B_\nu)}{A_\nu B_\nu} \to 1, \text{ when } A_\nu \to P,\ B_\nu \to P \text{ and } \frac{A_\nu P + PB_\nu}{A_\nu B_\nu} < M < \infty.$$

The neighborhood $XP < \rho$ coincides with $m(X, P) < \rho$. The metric $m(X,Y)$ may be extended to an internally and externally strictly convex metric space (which contains no new points in $m(X, P) < \rho$). One sees that this space admits a simply transitive Abelian group of motions. Now a theorem of Markoff[3] shows that $m(X, Y)$ is a Minkowskian Metric.

Detailed proofs will be forthcoming in a monograph on *Metric Methods in Finsler Spaces and Foundations of Geometry.*

[1] The proof is a generalization of H. Busemann, "Pasch'sches Axiom und Zweidimensionalität," *Math. Ann.*, **107**, 323–328 (1932).

[2] Examples show that Δ does not imply approximation in the sense of (2).

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INFINITE CYCLES AND HOMOLOGIES

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This note is concerned with the homology groups of an infinite complex. The main method will be that of group extensions. With this new tool, one can efficiently handle certain homology groups which previously seemed inaccessible.

1. Extensions of Groups. All groups to be considered are abelian. If G and H are given groups, a group E is said to be an *extension*[1] of G by H if E has G as a subgroup and $H = E - G$ as the corresponding difference

group. Two extensions E and E' will be regarded as equivalent if there is an isomorphism of E to E' in which both the elements of G and the cosets of H are left fixed. For fixed G and H, it is known[1] that the equivalence classes thus obtained form in natural fashion an abelian group, which we denote by $Ext\ \{G, H\}$. In this group of extensions, the zero element is determined by the direct sum, $E_0 = G + H$, regarded as an extension of G by H.

Each extension E of G by H induces an extension of G by H_0, for every subgroup H_0 of H. We consider those extensions E which induce the direct sum extension on every finite subgroup H_0 of H. They form a subgroup, $Ext_f\ \{G, H\}$, of the whole group $Ext\ \{G, H\}$.

2. Homomorphisms and Tensor Products. For given topological groups G and H, we let $Hom\ \{G, H\}$ stand for the group of all continuous homomorphisms of G into H, topologized in the well-known way. In the particular case when H is the group of reals modulo 1, the homomorphisms are called characters, and we write $Hom\ \{G, H\} = Ch\ G$. It is well known[2] that if G is compact (meaning bicompact), then $Ch\ G$ is discrete, and vice versa.

Whitney's tensor product[3] $G \circ H$ of two discrete groups can alternately be described as the group $Ch\ Hom\ \{H, Ch\ G\}$. If H is a finite group, the group $Ext\ \{G, H\}$ is naturally isomorphic to the tensor product $G \circ Ch\ H$.

In order to extend this result, we are led to a *modified tensor product* $G \bullet A$, defined for any group G and for a compact 0-dimensional group A. Let $\{A_\alpha\}$ be the family of all open and closed subgroups of A, ordered by inclusion. The groups $A - A_\alpha$ are finite and yield an inverse system[4] of homomorphisms. This yields a second inverse system $G \circ (A - A_\alpha)$, with a limit defined to be the new product $G \bullet A$.

3. Complexes and Homologies. Let K be an infinite complex which is locally finite, and which is assumed to be an abstract simplicial complex, as in Tucker's treatment[5] of finite complexes. The groups of chains, cycles and bounding cycles over a given coefficient group G are written as $C^q(K, G)$, $Z^q(K, G)$ and $B^q(K, G)$, respectively. Thus B^q consists of all chains of the form $d[\Sigma g_i \sigma_i^{q+1}]$, where d is the boundary operator, Σ is an infinite sum, each $g_i \in G$, and each σ_i is a $(q+1)$-cell. The coboundary operator δ and the whole cotheory is of the Whitney type. The groups of *finite* chains, finite cocycles and finite coboundaries are denoted respectively by $\mathfrak{C}_q(K, G)$, $\mathfrak{Z}_q(K, G)$ and $\mathfrak{B}_q(K, G)$. The qth *homology group* of infinite cycles of K with coefficients in G is then

$$H^q(K, G) = Z^q(K, G) - B^q(K, G).$$

The qth *cohomology group* of finite cocycles of K with coefficients in G is

$$\mathfrak{H}_q(K, G) = \mathfrak{Z}_q(K, G) - \mathfrak{B}_q(K, G).$$

The "torsion" subgroup of $\mathfrak{H}_q$ consisting of all the elements of finite order is denoted by $\mathfrak{T}_q(K, G)$.

In $Z^q(K, G)$ we also consider the subgroup $B_w^q(K, G)$ of the weak boundaries; $z^q \in B_w^q(K, G)$ if for each finite subset L of K there is a chain z_1^q in $K - L$ such that $z^q - z_1^q \in B^q(K, G)$. The *weak homology group* of infinite cycles is then

$$H_w^q(K, G) = Z^q(K, G) - B_w^q(K, G).$$

4. Statement of Results. The scalar product $c^q \cdot d_q$ of a chain $c^q = \Sigma g_i \sigma_i^q$ in $C^q(K, G)$ and a finite chain $d_q = \Sigma h_i \sigma_i^q$ with coefficients h_i in the additive group I of integers is defined as the element $c^q \cdot d_q = \Sigma h_i g_i$ of G. Relative to this scalar product we define

$$A^q(K, G) = \text{the annihilator of } \mathfrak{Z}_q(K, I).$$

We verify that

$$B^q(K, G) \subset B_w^q(K, G) \subset A^q(K, G) \subset Z^q(K, G).$$

In these terms we can state our main results:

(I) *$A^q(K, G)$ is a direct summand of $Z^q(K, G)$.*

(II) *$Z^q(K, G) - A^q(K, G)$ is isomorphic with the group Hom $\{\mathfrak{H}_q(K, I), G\}$ of all homomorphisms of the qth integral (finite) cohomology group into the coefficient group G.*

(III) *$A^q(K, G) - B^q(K, G)$ is isomorphic with the group Ext $\{G, \mathfrak{H}_{q+1}(K, I)\}$ of all group extensions of the coefficient group G by the $(q + 1)$-st integral (finite) cohomology group.*

(IV) *Under the isomorphism of* (III), *$B_w^q(K, G) - B^q(K, G)$ is mapped onto the group $Ext_f \{G, \mathfrak{H}_{q+1}(K, I)\}$.*

(V) *$A^q(K, G) - B_w^q(K, G)$ is isomorphic with the modified tensor product $G \bullet Ch\, \mathfrak{T}_{q+1}(K, I)$ of the coefficient group G by the character group of the torsion subgroup $\mathfrak{T}_{q+1}$ of the $(q + 1)$-st integral (finite) cohomology group.*

As corollaries we obtain the following direct sum decompositions of the homology groups

(VI) $H^q(K, G) \cong Hom\, \{\mathfrak{H}_q(K, I), G\} + Ext\, \{G, \mathfrak{H}_{q+1}(K, I)\}$.

(VII) $H_w^q(K, G) \cong Hom\, \{\mathfrak{H}_q(K, I), G\} + G \bullet Ch\, \mathfrak{T}_{q+1}(K, I)$.

5. Topologized Homology Groups. If G is topological, $C^q(K, G)$ carries a natural direct product topology which induces a topology on the group $H_w^q(K, G)$.[6] The isomorphism (II) is then topological. There is also a natural topology for the modified tensor product[6] so that the iso-

morphism (V) is also topological. Consequently the same holds for the isomorphism (VII).

Moreover, if G is compact then $B^q = B^q_w$, $H^q = H^q_w$ and

$$\text{(VIII)}\quad G \bullet Ch\ \mathfrak{T}_{q+1}(K, I) \cong Ch\ Hom\ \{G,\ \mathfrak{T}_{q+1}(K, I)\} \cong Ch\ Hom\ \{Ch\ \mathfrak{T}_{q+1}(K, I),\ Ch\ G\}.$$

Using the duality theorem which asserts that $\mathfrak{H}_q(K, G) \cong Ch\ H^q_w(K, Ch\ G)$ for G discrete, we obtain an expression for $\mathfrak{H}_q(K, G)$ in terms of G, $\mathfrak{H}_q(K, I)$ and $\mathfrak{T}_{q+1}(K, I)$.[7]

The assumption that the complex is locally finite can be replaced by the weaker assumption of star-finiteness for most of the results. Analogous theorems are obtained interchanging homology and cohomology and replacing $q + 1$ by $q - 1$ throughout.

6. Compact Metric Spaces. To treat a compact metric space X we consider the fundamental complex[8] K of X and the relations of its homology groups to those of X. Steenrod[9] has introduced the homology group $H^q(X, G)$ of regular cycles in the space X and has shown that

$$H^q(X, G) \cong H^q(K, G).$$

Also, it is well known that the Čech homology and cohomology groups $\mathfrak{H}^q(X, G)$ and $\mathfrak{H}_q(X, G)$ are, respectively, isomorphic with $H^{q+1}_w(K, G)$ and $\mathfrak{H}_{q+1}(K, G)$. Therefore substituting in the previous theorems we get the following results:

(i) $H^q(X, G)$ is determined by G and the integral cohomology groups $\mathfrak{H}_{q-1}(X, I)$ and $\mathfrak{H}_q(X, I)$ of the space,

(ii) $\mathfrak{H}^q(X, G)$ is determined by G and the integral cohomology group $\mathfrak{H}_q(X, I)$ and the torsion group $\mathfrak{T}_{q+1}(X, I)$, where the groups and the isomorphisms involved are all topological.[10]

(iii) $H_q(X, G)$ is determined by G and the groups $H_q(X, I)$ and $\mathfrak{T}_{q+1}(X, I)$.

Steenrod also introduces the homology group $\tilde{H}^q(X, G)$ of those regular cycles which are sums of finite cycles, and represents it in terms of the fundamental complex as $B^q_w(K, G) - B^q(K, G)$. Hence

$$\tilde{H}^q(X, G) \cong Ext_I\ \{G, \mathfrak{H}_q(X, I)\}.$$

Steenrod's computation of this group for the case of the p-adic solenoid suggested to us the application of group extensions.

7. Construction of Group Extensions. The essential device for connecting group extensions with homology groups is the following. Let z^q

be a fixed chain in $A^q(K, G)$. In the direct sum $\mathfrak{Z}_{q+1}(K, I) + G$, the elements of the form $\delta d_q - z^q \cdot d_q$, for all chains d_q, constitute a subgroup S isomorphic with $\mathfrak{B}_{q+1}(K, I)$. The difference group $(\mathfrak{Z}_{q+1} + G) - S = E(z^q)$ is a group extension of G by the cohomology group $\mathfrak{H}_{q+1}(K, I) = \mathfrak{Z}_{q+1} - \mathfrak{B}_{q+1}$.

[1] Zassenhaus, H., *Lehrbuch der Gruppentheorie*, Berlin, 1937, pp. 89–98.

[2] Pontrjagin, L., *Topological Groups*, Princeton, 1939, Ch. V; v. Kampen, E. R., *Ann. Math.*, **36**, 448–463 (1935).

[3] Whitney, H., *Duke Math. Jour.*, **4**, 495–528 (1938).

[4] Steenrod, N. E., *Amer. Jour. Math.*, **58**, 661–701 (1936).

[5] Tucker, A. W., *Ann. Math.*, **34**, 191–243 (1933).

[6] This topology will be a Hausdorf topology if G is a division closure group (Steenrod, loc. cit., p. 676).

[7] Čech, E., *Fund. Math.*, **25**, 33–44 (1935).

[8] Lefschetz, S., *Topology*, New York, 1930, p. 325.

[9] Steenrod, N. E., *Ann. Math.*, **41**, 833–851 (1940).

[10] This is essentially the Steenrod universal coefficient theorem for compact metric spaces.

SOME PROPERTIES OF REAL LINEAR TOPOLOGICAL SPACES

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We denote a real linear topological space, l. t. s., by the symbol L and adopt Hyers'[1] slight modification of the von Neumann axioms[2] for the characterization of such a space. If $\mathfrak{N} = \{N\}$ is a neighborhood basis of the origin,[3] θ, in the l. t. s. it is easy to show that the sets $U = \{\lambda x \mid 0 \leqslant \lambda \leqslant 1, xeN\}$ constitute an equivalent neighborhood basis. We denote the closed hull of A by $\overline{A}$, the set of inner points by $\underline{A}$ and the complement by $\tilde{A}$. The symbol ϕ stands for the null set. The set of all linear functionals on L is designated by L^* with null element θ^*. We shall say A encircles θ if every half ray from θ has an interval containing θ in common with A.

1. Preliminary Results.

LEMMA 1: *Every U encircles θ.*

LEMMA 2: *If A is closed and $x_0 e \tilde{A}$ then for some U_0, $(A + \overline{U}_0) \cap x_0 = \phi$.*

LEMMA 3: *If K is convex then $\overline{K}$ and $\underline{K}$ are convex.*

LEMMA 4: *If K is convex, $\tilde{K} \neq \phi$ and $\theta e \underline{K}$ there is a triangular pseudo norm which satisfies* (1) $0 \leqslant \|x\| < \infty$, $\|\theta\| = 0$; (2) $\|x\| \leqslant 1$ for xeK; (3) $\|tx\| = t\|x\|, t \geqslant 0$; (4) $\|x_1 + x_2\| \leqslant \|x_1\| + \|x_2\|$.

If for every $x_0 \bar{e} K$ there is an $f_0 eL^*$ such that $f_0(x_0) > \sup_{xeK} f_0(x)$ we shall say

K is *regularly L convex.* It is easy to show that regular L convexity implies convexity.

THEOREM 1. *If $K \neq L$ is convex and closed and $\underline{K} \neq \phi$ then K is regularly L convex.*

2. The Weak Topology of L. The weak neighborhoods in L and L^* are defined in the usual way. The weak topology of L as elements and of L^* as functionals is indicated by the symbols $w(L)$ and $w^*(L^*)$, respectively. In general it is not true that L is a l. t. s. in its $w(L)$ topology.

Let $\Theta = \{x \mid f(x) = 0 \text{ for all } feL^*\}$. Let $X = x + \Theta$. The sets Θ, X considered as elements of a new space J are designated by $\mathbf{O}, \mathbf{X}$, respectively. The neighborhoods of $\mathbf{O}$ in J are defined by $\mathbf{V} = \{\mathbf{X} \mid XeW\}$ where W is a $w(L)$ neighborhood of θ. Evidently J is a space of disjunct equivalence classes defined on L. Moreover Θ is plainly convex and hence kx_1 and $x_1 + x_2$ correspond to $k\mathbf{X}_1$ and $\mathbf{X}_1 + \mathbf{X}_2$ where $x_i eX_i$, $i = 1, 2$, so that J is a linear space. The transformation from L to J is connoted by writing $\mathbf{X} = Tx$.

THEOREM 2. *The space J is a completely regular, Hausdorff, locally convex l. t. s.*

The product space $\mathbf{L}_{1/4} \times R$, where $\mathbf{L}_{1/4}$ is the space $\mathbf{L}_p$, $p = 1/2$, and R is the real line is an example of a l. t. s. for which $L \neq \Theta \neq \theta$. Some properties of Θ are stated in the next theorem.

THEOREM 3. *The set Θ (a) contains no inner point if L admits a non null linear functional; (b) is entirely within either K or $\tilde{K}$ where K is convex, closed and $\underline{K} \neq \phi$; (c) is θ if L is locally convex.*

THEOREM 4. *If A is $w(L)$ closed it is closed.*

THEOREM 5. *The following conditions on K are equivalent:* (a) *K is regularly L closed;* (b) *K is convex and $w(L)$ closed;* (c) *$T(K)$ is convex and $w(J)$ closed.*

3. Local Convex Spaces. For the results in this section it is understood that L is locally convex.

LEMMA 5: *If A is bounded then $\overline{A}$ is bounded.*

LEMMA 6: *If K is closed and convex and $x_0 \bar{e} K$ then there is a closed convex set K^1 with $\underline{K}^1 \neq \phi$ such that $\tilde{K} \supset \tilde{K}^1 \supset x_0$.*

THEOREM 6. *A locally convex l. t. s. is a locally convex completely regular l. t. s. in its $w(L)$ topology.*

THEOREM 7. *If K is convex and closed then K is $w(L)$ closed.*

This extends a classic result due to Mazur.[4]

4. Metrizability of L*. We denote by $B \subset L$ a bounded set containing θ. If there is a fixed sequence of such sets, $\{B_n\}$, with the property that any B is interior to some $B_n e \{B_n\}$ we shall say that L admits an *outer countable basis.* The neighborhoods of θ^* are given by $U^* = \{f \mid |f(x)| \leqslant 1, xeB\}$. The notion of an outer countable basis and the first part of Theorem 8 were suggested to the writer by Professor Kakutani in the course of a conversation.

THEOREM 8. *A sufficient condition for the metrizability of L^* is that L admits an outer countable basis. If L is locally convex the condition is also necessary.*

5. Local Boundedness. If L contains a bounded open set Q then L is locally bounded. Hyers has shown that in this case there is a pseudo norm satisfying (1) $\|x\| \geqslant 0$ and $\|x\| = 0$ implies $x = \theta$; (2) $\|tx\| = |t|\,\|x\|$; (3) for every $\eta > 0$ there is a $\delta > 0$ such that $\|x + y\| < \eta$ whenever max. $(\|x\|, \|y\|) < \delta$; (4) the sets $\{x \mid \|x\| < \epsilon, \epsilon > 0\}$ constitute a neighborhood basis equivalent to $\mathfrak{U}$; (5) for arbitrary x and $\eta > 0$ there is a $\delta > 0$ such that $\|y\| - \|x\| < \eta$ for $\|y - x\| < \delta$. We show below that conditions (3) and (5) may be made significantly sharper, with the consequence that the pseudo norm can be used in many arguments depending on estimates of upper bounds, in a similar way to that in which the norm enters in vector normed spaces.

THEOREM 9. *If L is locally bounded the pseudo norm satisfies $\|x_1 + x_2\| \leqslant a(\|x_1\| + \|x_2\|)$ for some a independent of x_1 and x_2.*

The lack of continuity of the pseudo norm for the general locally bounded l. t. s. is brought into evidence in the following corollary:

COROLLARY: *If x^n converges to x_0 then $a\|x_0\| \geqslant \limsup \|x^n\| \geqslant \liminf \|x^n\| \geqslant \|x_0\|/a$.*

If P is a linear operator on L_1 to L_2 where L_1 and L_2 are locally bounded then $\|P\|$ is defined as $\sup_{xeQ^1} \|P(x)\|_2$ where $Q^1 = \{\lambda x \mid -1 \leqslant \lambda \leqslant 1, xeQ\}$. The space of such operations is designated by $\mathbf{P}$.

THEOREM 10. *The pseudo norm in $\mathbf{P}$ satisfies $\|P_1 + P_2\| \leqslant a_2(\|P_1\| + \|P_2\|)$ where a_2 is the constant a associated with L_2.*

If L_2 is a normed vector space then $\mathbf{P}$ is, also.

6. The Conjugate Space L^*. The concept of *regular L^* convexity* for K is defined by the requirement $f_0(x_0) > \sup_{feK} f(x_0)$.

THEOREM 11. *The statements* (a) *K is regularly L^* convex and* (b) *K is convex and $w^*(L^*)$ closed, are equivalent.*

Many results concerned with the w^* topology of the conjugate space of a vector normed space have valid analogues when L is a locally bounded l. t. s. The following results are of this type.

THEOREM 12. *If L is locally bounded then the unit sphere S^*, in L^*, is bicompact in the $w^*(L^*)$ topology.*

LEMMA 7: *If L is locally bounded, then, if $K \subset L^*$ is either $w^*(L^*)$ closed, regularly L^* convex or transfinitely closed,[5] K is closed.*

THEOREM 13. *If L is locally bounded and $K \supset L^*$ is bounded then a necessary and sufficient condition that K be $w^*(L^*)$ closed and convex is that K be transfinitely closed.*

The writer has not as yet settled the question of the validity of Theorem 13 when the boundedness condition on K is waived. LaSalle[6] has given a

criterion for the existence of a non null linear functional on a l. t. s. This criterion may be extended in scope.

THEOREM 14. *A necessary condition for the existence of a non null linear operator on L_1 to a normed vector space L_2 is that L_1 properly contains an open, convex set.*

The proof depends on a specialization of the following lemma.

LEMMA 8: *If P is a linear operator on L_1 to L_2 and P^1 is the associated linear operator on L_2^* to L_1^* then $P(\Theta_1) \subset \Theta_2$. If L_1 and L_2 are locally bounded then $\|P\| \leqslant \|P^1\|$.*

7. Banach Spaces.[5] In this section L is always a Banach space, S is the unit sphere and German capitals mean directed sets. We use the definition of Alexandroff and Urysohn[7] for bicompactness of order $\aleph_a$ and the phraseology $w(L)$ *sequentially compact* implies $w(L)$ *sequentially closed.* If A is $w(L)$ closed the *sections* $A \bigcap \{x | f(x - x_0) \geqslant \epsilon, x_0 \epsilon \bar{A}\}$ constitute a subbasis for the $w(L)$ closed sets in A. We may introduce $w(L)$ *sectional* bicompactness by requiring the closed sets entering into the bicompactness definition to be sections. It may be shown, however, that $w(L)$ sectional bicompactness is equivalent to $w(L)$ bicompactness. We define $w(L)$ completeness of order $\aleph_a$ by requiring the directed sets in Goldstine's[8] definition of $w(L)$ completeness to have power $\aleph_a$.

THEOREM 15. *If S is $w(L)$ bicompact of order $\aleph_a$, it is $w(L)$ complete of order $\aleph_a$.*

Besides the weak topologies in L and L^* we may introduce weak convergence topologies by requiring a *convergent* function on a directed set to be uniformly bounded.[9] For bounded sets the $w(L)$ ($w^*(L^*)$) topologies and $w(L)$ ($w^*(L^*)$) convergence topologies are equivalent. In particular this is true for $w(L)$ bicompact sets of any order since such sets are easily shown to be bounded. The equivalence of the topologies mentioned is lost when unbounded sets may enter. For instance von Neumann has given an example of an *unbounded* sequence with a $w^*(L^*)$ limit which is not associated with any $w^*(L^*)$ convergent subsequence.[10] (In this connection it is of interest to note that the writer has obtained an example of a bounded and even $w^*(L^*)$ bicompact set with no $w^*(L^*)$ sequential limits.) The possible generalizations of theorems involving weak sequential convergence must take into account the influence of unbounded sets. The two types of extensions, below, of Banach's Theorem 2, p. 213, illustrate this point.

THEOREM 16. *Let Γ be a linear subspace of L^*. A necessary and sufficient condition for $\bar{\Gamma} = L^*$, where $\bar{\Gamma}$ is the $w^*(L^*)$ closure of Γ, is that for arbitrary $x, f(x) = \|x\|$ for some $f \epsilon \Gamma$. If $\bar{\Gamma}$ stands for the $w^*(L^*)$ convergence closure then this $f \epsilon \Gamma$ can be chosen so that $\|f\| \leqslant M$.*

A set A in L has property Q if whenever $\{f^\mu | \mu \epsilon \mathfrak{A}\}$ is $w^*(L^*)$ convergent to θ^* then for arbitrary $\mu \epsilon \mathfrak{A}$ and $\epsilon > 0$ there is a finite set $\mu_{j,\bar{\mu}}$; $j = 1, \ldots N_{\bar{\mu}}$ such that $\mu_{j,\bar{\mu}} > \bar{\mu}$ and $A \subset U_{j=1}^{N_{\bar{\mu}}} W(\theta^*; f^{\mu_{j,\bar{\mu}}}; \epsilon)$ where the notation for

the weak neighborhood W carries its customary interpretation. Evidently $w(L)$ bicompact sets have property Q.

THEOREM 17. *If $A, A \subset L$, has property Q (or is $w(L)$ bicompact) and if (a) $\{x^a | a \epsilon \mathfrak{A}\} \subset A$ converges $w^*(L^{**})$ to $X_0 \epsilon L^{**}$ and (b) $\{f^b | b \epsilon \mathfrak{B}\}$ converges $w^*(L^*)$ to $f_0 \epsilon L^*$ then $L_a L_b f^b(x^a) = L_b L_a f^b(x^a) = X_0(f_0)$, where the limits are taken in the Moore-Smith sense.*

THEOREM 18. *If A has property Q (or is $w(L)$ bicompact) and $\{f^\mu\}$ is dense in the $w^*(L^*)$ convergence topology then the sets $A \bigcap \{x | f^\mu(x) \geqslant \delta\}$ form a subbasis for the sets $\{x | f(x) \geqslant \eta\} \bigcap A$ where f is arbitrary in L^* and δ and η are unrestricted.*

This result is useful in establishing theorems for Banach spaces by arguments which are essentially topological in nature. The next theorem, for instance, can be proved in this way.

THEOREM 19. *If S is $w(L)$ sectionally bicompact of order $\aleph_a$ and L^* admits a dense set of power $\aleph_a$ in its $w^*(L^*)$ convergence topology, then L is reflexive.*[11]

We shall say L has the *Helly property*[12] *of order* $\aleph_a$ if under the condition $|\sum_{i=1} \lambda_{\mu_i} C_{\mu_i}| \leqslant M \| \sum_{i=1} \lambda_{\mu_i} f^{\mu_i} \|$, where the λ_{μ_i}'s are any finite set of real numbers, the system $f^\mu(x) = C^\mu$, with $\{\mu\}$ of power $\aleph_a$, has a solution $x_\epsilon \epsilon (M + \epsilon) S$ for each $\epsilon > 0$. Some typical theorems follow.

THEOREM 20. *If S is $w(L)$ bicompact of order $\aleph_a$ then L has the Helly property of order $\aleph_a$.*

THEOREM 21. *If L has the Helly property of order $\aleph_a$ and L^* contains a total set of power $\aleph_a$ then (a) S is $w(L)$ complete (b) L is reflexive (c) S is $w(L)$ bicompact of order $\aleph_a$.*

We now indicate some relations between various compactness notions.

THEOREM 22. *Norm compactness in L^* implies $w^*(L^*)$ bicompactness.*

THEOREM 23. *If L is separable and $A, A \subset L$, is $w(L)$ sequentially compact, then A satisfies the second countability axiom of Hausdorff, in its $w(L)$ topology.*

THEOREM 24. *If $A, A \subset L$, is $w(L)$ bicompact of any order then A is $w(L)$ sequentially compact. If A is $w(L)$ sequentially compact then A is $w(L)$ bicompact of order $\aleph_0$.*

A complete discussion together with detailed proofs of the results of this note will be published elsewhere.

[1] Hyers, D. H., "Locally Bounded Linear Topological Spaces," *Revista de Ciencas*, **41**, 558–574 (1939).

[2] von Neumann, J., "On Complete Topological Spaces," *Trans. Am. Math. Soc.*, **37**, 1–20 (1935).

[3] Several types of neighborhoods of the origin are introduced in the course of this paper. It is tacitly understood that a neighborhood of an arbitrary element is always to be obtained by vector addition of that element to those constituting the neighborhood of the origin.

[4] Mazur, S., "Uber konvex Mengen in linearen normierten Raume," *Studia Mathematica*, **4**, 70–84 (1933).

[5] Banach, S., *Theorie des Operations Lineares* (1932).

[6] LaSalle, J. P., "Pseudo Normed Linear Spaces," *Duke Math. Jour.*, **8**, 131–135 (1941).

[7] Alexandroff, P., and Urysohn, P., "Zur Theorie der Topologische Raume," *Mathematische Annalen*, **92**, 258–266 (1925).

[8] Goldstine, H. H., "Weakly Complete Banach Spaces," *Duke Math. Jour.*, **4** (1938).

[9] Alaoglu, L., "Weak Topologies of Normed Linear Spaces," *Ann. Math.*, **41**, 253–267 (1940).

[10] von Neumann, J., "Zur Algebra der Funktional Operationen," *Mathematische Annalen*, **102**, 370–427 (1929).

[11] The definition of the Helly property of order $\aleph_a$ and theorems 19–21 were presented (among others) to the American Mathematical Society at the May and September meetings, 1941 At the latter meeting M. Day announced similar results and R. S. Phillips who had been apprised of the writer's theorems, presented, in part, different proofs of these results.

[12] This generalizes a concept introduced by Helly. A simple proof of Helly's theorem is given in Kakutani, S., "Weak Topology and Regularity of Banach Spaces," *Proc. Imp. Acad., Tokio*, **15**, 169–173 (1939).